

Survival and Control of Viability of Seeds with Special Emphasis on Tropical Species

INAUGURAL-DISSERTATION

submitted to
the Faculty of Philosophy II (Natural Sciences)
of the University of Zurich
for the degree of Doctor of Philosophy

by

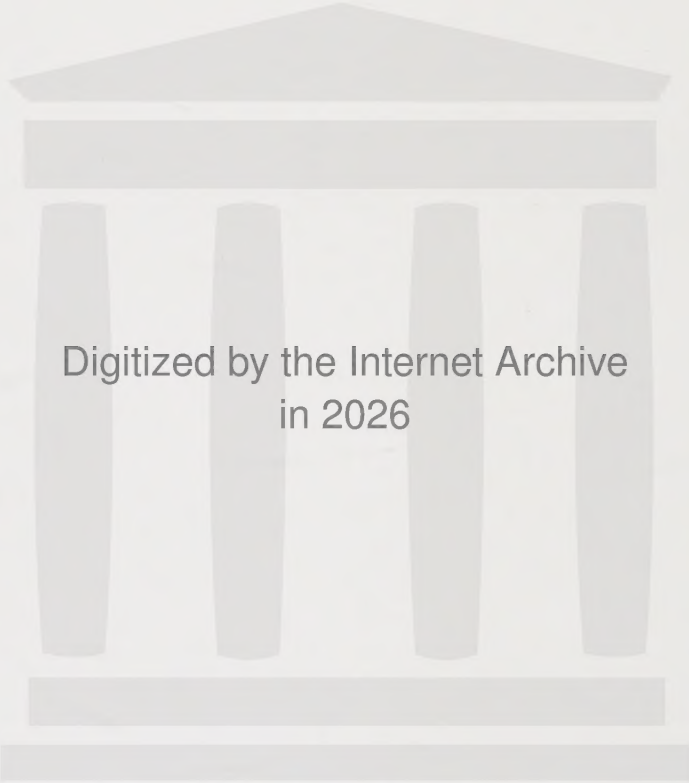
HADI SUTARNO
Citizen of the Republic Indonesia

Approved by
Prof. Dr. H. Wanner

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BRUNNEN, NUREMBERG

1971

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- sapa kang nandur wijining kabecikan
bakal ngunduh kabecikan uga,
ananging ...
kang dadi tantangan
kepiye ngayomi ajining wiji
saka pudaring zaman

- who grows a seed of goodness
he will harvest a goodness too,
but ...
the challenge is
how to protect a quality of seed
from the degradation by time

- Dedicated to:
my wife dik S.A. Purbowardhani
and people who love the young generation.

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1. INTRODUCTION

A seed is a unit consisting of embryo and ancillary organs that are excellently adapted to fulfill their biological functions – the conservation and dissemination of species. Seeds are also of great significance in the life of man. They are an important source of nutrition as well as of many valuable medicinal and industrial substances. The successful cultivation of useful plants largely depends on the quality of seeds – especially viability and germination vigor.

A number of scientists spent a long time studying the problem of seed dormancy; Nikolaeva (1969) attempted to study physiology of deep dormancy in seeds. Recently there appeared several reviews and investigations of the problems of seed dormancy and germination (Vegis, 1964; Barton 1965a, 1965b; Evenari, 1965; Lang, 1965; Wareing, 1965; Chatterji & Mohnot, 1968; Pandey, 1968; Maun & Cavers, 1971; Willemsen, 1975; Baskin & Baskin, 1977; Vincent & Cavers, 1978). Nevertheless, many very essential phenomena of seed physiology remain obscure.

The most fundamental problem among the various aspects of seed science is seed viability. Very little information is available on seed viability of tropical species. Recently the need for increased study on seed viability has been stressed by workers in the fields of exploration, conservation and utilization of plant genetic resources. The problems of seed viability are relevant in a number of applied fields. Seed producers are interested in the factors affecting viability before harvest. Farmers, agronomists and horticulturists hit on viability problems after sowing. For seedmen the problem of maintaining viability in storage is essential. Breeders and conservators of genetic material have a special interest in long-term storage systems. Furthermore the survival of seed

populations of wild species in the crop soil is of interest to plant ecologists and especially to those concerned with practical problems of weed control. In the technology of grain storage for food it has long been known that percentage viability is a good criterion of nutritional grain quality (Oxley, 1948).

Both on a world-wide and smaller ecological scale, plant distribution is strongly influenced by ambient temperatures, because plants are not able to regulate their temperature effectively. Since seed is more protected than other stages of development in the life cycle, longevity, germination vigor and seedling development are important for a strategy towards successful competition. For instance it has been reported that the family Caryophyllaceae shows a pattern of germination response to temperature which appears to be correlated with the geographical distribution of species, and which allows seedling establishment to occur at the most favourable time of year in their particular climatic area (Villiers, 1975). Further work (Thompson, 1970) on germination pattern in a wide temperature range shows clearly a relation between germination behavior and plant distribution or climate where the species occur. For most species there is still little or no information about the suitable temperature regimes for germination and seedling development. Such information could be useful for the interpretation or control of plant distribution and their survival.

In nature temperature is never constant, but varies with the diurnal and seasonal fluctuation of climatic factors. Altitude and geographical latitude of the region are most influential for the temperature variations. It has long been known for some species that the mean temperature, but also frequency and amplitude of temperature variations during germination may determine the successful establishment of a species.

Every seedling establishment in a plant association gives the plant a chance of competition among the members of the community. Similarity of germination behavior is effective for the start of competition. For example wild oat, Avena fatua, germinates competitively in wheat fields sown in spring. In an other situation the wild oat, Avena ludoviciana, germinates competitively in wheat fields sown in autumn (Hill, 1977). In this case climatic factors as temperature and water besides the soil seed bank are important as variables in seedling establishment.

The temperature regime may also determine seed production as shown for Juncus squarrosus. The occurrence of this species on British mountains is limited by its failure to produce viable seeds above about 900 m, although the plants will grow vegetatively up to 1200 m (Sutcliffe, 1977). A similar phenomenon is shown by Machaerina rubiginosa, Eleocharis dulcis and Scirpus juncoides, in Java common in lowland, occurring in higher altitudes only in sterile state, propagating vegetatively. In contrast Albizia lophantha of which the main altitudinal range is 1700 - 3100 m occurs exceptionally down to 1100 m but then remains sterile (Backer & Brink, 1965). Many plants are unable to survive in deserts because of their inability to withstand the maximum temperatures during the hottest periods.

It has long been known that plants produced from old seeds produce a higher proportion of phenotypic variants than those from fresh seed. A number of workers reported increased frequencies of chromosome aberrations with increase in age of seed from a wide range of species: e.g. in Allium cepa (Nichols, 1941; Sax & Sax, 1964) in Allium fistulosum, Triticum aestivum, Hordeum distichon, Secale cereale and Pisum sativum (Gunthardt, Smith, Haferkamp & Nilan, 1953). Evidence that factors such as temperature and mois-

ture during storage are important in this respect came from cytological work on Crepis (Navashin & Gerassimova, 1936a, b) and from investigations on pollen abortion in Datura (Cartledge, Barton & Blakeslee, 1936). The authors concluded that 'in general the mutation rate increased with increased moisture content, and with increased duration of treatment'. It is known that farmers or seed producers need no long-term storage of seeds. Every year they regenerate their seeds, so the accumulation of mutations during storage is avoided. In contrary seedmen, breeders and conservators of genetic material who need long-term storage of seeds, found that sometimes degradation of genetic quality or genetic erosion by storage is alarming, because of the accumulation of mutations associated with loss of viability (Roberts et al, 1967; Abdalla & Roberts, 1968, 1969). Therefore seeds in storage must be regenerated in certain time intervals. The duration of these intervals depends on genetic factors and the storage conditions. It has been suggested that at present best estimates of regeneration interval are obtained by applying the three viability equations and the four viability constants determined at higher temperatures (Roberts & Ellis, 1977). With this procedure it is possible to predict the time taken for viability to fall by 5% at a standard storage temperature of -6°C and seed moisture content of 5%.

In this work I intended to provide information on the cardinal temperatures of germination for a number of tropical plant species. I had the special opportunity to work with many species from Java. They were selected from different natural areas of distribution, namely from the lowland to altitudes up to 3000 m and from the wet west part to the dry eastern part of the island. I attempted to correlate the seed's temperature characteristics with the geographical and altitudinal distribution.

Another aspect of seed physiology I studied was seed longevity. For this purpose I adopted the technique of artificially ageing the seed (Roberts & Ellis, 1977).

2. MATERIALS AND METHODS

2.1. Material

All plant names and taxonomic relations are adopted from Backer and Brink 'Flora of Java' (1963, 1965). The geographical distribution of the Javanese species investigated has been checked with material from the Herbarium Bogoriense, Indonesia.

Dry seeds either from fields or purchased were cleaned before using for experiments. They were placed in open containers at room temperature (20-25°C) for 7-10 days, and then stored in sealed containers at 14°C in the dark. The materials included (Table 1).

Table 1: Species, date and region of collection

Species	Collection *
A. Non cultivated	Date, lacion and altitude (m)
<u>Ageratum conyzoides</u> L. (Asteraceae)	July 78, J (113)
<u>Albizia lophantha</u> (Willd.) Bth. (Mimosaceae)	July 78, G-P (2400)
<u>Amaranthus spinosus</u> L. (Amaranthaceae)	July 78, J (113)
<u>Borreria brachystema</u> (R. Br. ex Bth. Valet. (Rubiaceae)	July 78, J (113)
<u>Cassia tora</u> L. (Caesalpiniaceae)	July 78, T (950)
<u>Cleome gynandra</u> L. (Capparidaceae)	July 78, J (113)

(continued)

	Collection *
	Date, location and altitude (m)
<u>Crotalaria mucronata</u> Desv. (Papilionaceae)	July 78, J (113)
<u>Elaeagnus conforta</u> Roxb. (Elaeagnaceae)	July 78, G-P (1350)
<u>Hypericum leschenaultii</u> Choisy. (Hypericaceae)	July 78, G-P (3015)
<u>Hyptis brevipes</u> Poit. (Lamiaceae)	July 78, J (113)
<u>Impatiens javensis</u> (Bl.) Steud. (Balsaminaceae)	January 79, G-P (2130)
<u>Mycetia cauliflora</u> Reinw. (Rubiaceae)	July 78, G-P (1500)
<u>Myrsine affinis</u> DC. (Myrsinaceae)	July 78, G-P (2760)
<u>Polygonum chinense</u> L. (Polygonaceae)	December 78, G-P (2130)
<u>Taraxacum officinale</u> Wiggers. (Asteraceae)	July 78, T (950) June 78, V (1175)
B. Cultivated	
<u>Amaranthus caudatus</u> L. (Amaranthaceae)	April 78, purchased from SM
<u>Amaranthus paniculatus</u> L. (Amaranthaceae)	October 77, purchased from SM
<u>Amaranthus tricolor</u> L. (Amaranthaceae)	October 77, B (250)
<u>Cajanus cajan</u> (L.) Huth. (Papilionaceae)	May 78, P (75)

(continued)

Collection *

Date, location and
altitude (m)

Capsicum annuum L.
(Solanaceae)

May 78, B (250)

Celosia argentea f. plumosa L.
(Amaranthaceae)

November 78, pur-
chased from SM

Leucaena leucocephala (Lmk.)
De Wit (Mimosaceae)

January 78, P (75)

Ocimum americanum L.
(Lamiaceae)

July 78, J (113)

Phaseolus aureus Roxb.
(Papilionaceae)

December 77, pur-
chased from P & S

Phaseolus pubescens Bl.
(Papilionaceae)

May 78, P (75)

Sesamum orientale L.
(Pedaliaceae)

October 77, P (75)

Sesbania bispinosa (Pers.)
Fawc. & Rendle (Papilionaceae)

October 77, purchased
from P & S

Talinum paniculatum (Jacq.)
Gaertn. (Portulacaceae)

October 78, T (950)

Vigna unguiculata (L.) Walp.
(Papilionaceae)

May 78, P (75)

*)

B : Bogor, Java

G-P : Gede-Pangrango mountain, Java

J : Jogjakarta, Java

P : Purwokerto, Java

P & S : Pratap Nursery & Seed Store, Dehra Dun, India

SM : Samen Mauser, Zurich, Switzerland

T : Tawangmangu, Java

V : Valle, Airolo, Tessin, Switzerland

2.2. Criterion of germination

In this work, "germination in normal condition" means germination which is carried out in a constant temperature room of 26°C , under light for 15 hours everyday. In general two layers of wetted S. & S. no. 595 filter-paper were used in experiments. During germination, more distilled water was added if necessary.

The 2 mm emergence of the radicle through the seed coat was chosen as the criterion for germination in all experiments. Percentage germination was recorded everyday by counting and removing germinated seeds from petri dishes or plates of germination.

2.3. Germination at constant temperature

Germination was carried out in the 37 compartments of a thermo-gradient bar apparatus, under continuous light of 50 lux from fluorescent lamps. The temperature gradient extends from 0°C - 50°C with 0.5°C - 4.0°C steps from the first to the 37th compartment; temperature fluctuations of each compartment being about 0.8°C .

Ten to fifty seeds depending on seed size were germinated in each compartment. Seeds were sown on 3 layers of filter-paper strips. Water lost through evaporation in the warm compartments was replaced by filter-paper wicks leading to water in the vials placed at both ends of the gradient bar. Everyday for 25 days during germination the germinating seeds were recorded. The successive days for 50% germination were plotted against the temperature of germination. The temperature corresponding to the least number of days for 50% germination is considered to be the optimum temperature of germination (Fig. 1).

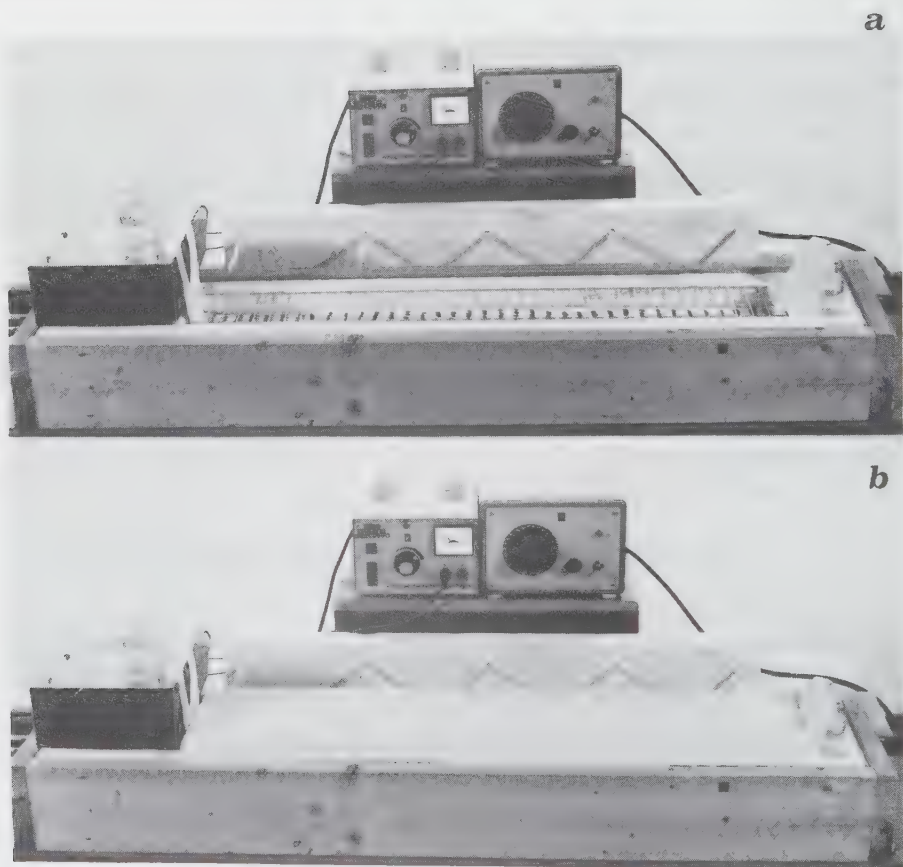


Fig. 1: The thermogradient bar apparatus; photo (a), the compartments for germination are covered with glass plates and a sheet of isolating material in photo (b). The temperature gradient extends from 0°-50°C with 0.5°-4.0° steps from the first to the 37th compartment. Water lost through evaporation in the warm compartments was replaced by filter-paper wicks leading to water in the vials placed at both ends of the apparatus. Peltier (cooler, left side) and heating elements (right side) are used for cooling and heating of the compartments of germination.

2.4. Germination at alternating temperature

Thirty or fifty seeds were germinated on 2 layers of filter-paper in petri dishes, diameter 9 cm. Seeds were moistened with 3 ml distilled water. Alternating temperature germinations were performed at $10/17^{\circ}$, $17/22^{\circ}$, $22/30^{\circ}$ and $10/30^{\circ}\text{C}$. Seeds were transferred from one incubation temperature to its alternative once every 12 hours. Samples with 4 or 5 replicates were germinated in continuous darkness for 10 days. Percentage germinations were recorded in a dark room under light of 25 W OSRAM lamp through a Kodak Safety filter no. 7 (dark-green). Response to alternating temperatures on germination was compared with results of germination at constant temperatures of 10° , 17° , 22° and 30°C . Differences were statistically tested by the t-test.

2.5. Influence of a pre-imbibition on germination at alternating and constant temperatures

Materials, equipment and time of germination were the same as in experiment 2.4. Seeds were soaked first for 5 hours at 26°C and continuously illuminated by 500 lux from fluorescent lamps. They were then transferred to a temperature regime of 10° , $10/17^{\circ}$, 17° , $17/22^{\circ}$, 22° , $22/30^{\circ}$, 30° and $10/30^{\circ}$. The influence of pre-imbibition on germination was determined by comparing these results with the results of germination without pre-imbibition at the same temperatures.

In another experiment, the influence of light during pre-imbibition was investigated.

2.6. Effect of chilling at 10°C on germination

Samples consisting of 30 or 50 seeds with 4 or 5 replicates were moistened with 3 ml distilled water in petri dishes of 9 cm diameter on 2 layers of filter-paper.

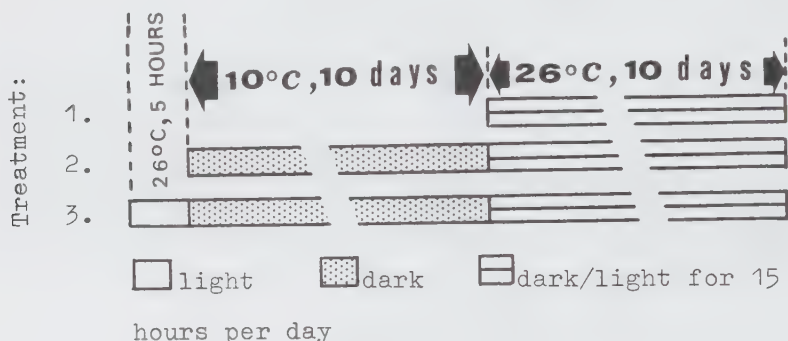


Fig. 2: Diagram of the experiment 2.6.

The petri dishes with the seeds to be chilled were packed in black plastic bag in moist conditions and kept in a refrigerator at 10°C for 10 days. After chilling the seeds were transferred to germinate for 10 days in a room at 26°C and illuminated with 500 lux from fluorescent lamps for 15 hours per day (treatment 2). This treatment was compared with treatment 1 without chilling pretreatment.

In treatment 3 the seeds were allowed to imbibe for 5 hours in light at 26°C before being chilled as in treatment 2.

2.7. Effect of chilling at various temperatures on germination

Samples consisting of either 10, 20, 30 or 50 seeds depending on seed size, were chilled in compartments of the thermogradient bar

apparatus at temperatures of 2° - 10°C on 3 layers of moistened filter-paper strips for 15 days, under continuous light of fluorescent lamps (50 lux). After chilling they were transferred to germinate in 9 cm petri dishes in normal conditions for 10 days.

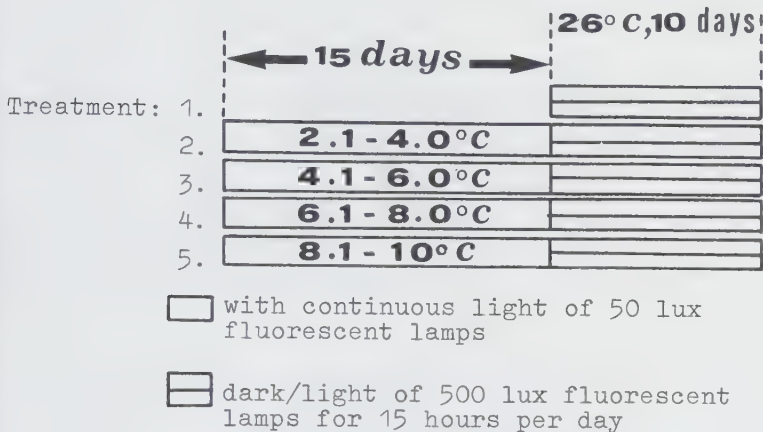


Fig. 3: Diagram of the experiment 2.7.

2.8. Heat sensitivity

Samples consisting of either 30 or 50 seeds depending on seed size, were sown on two layers of S. & S. no. 595 filter-paper of diameter 7 cm on the plate of the germination apparatus no. 985/90 from Se-madeni AG, Ostermundigen (Fig. 4). By means of a filter-paper wick the seeds are constantly kept moist. Heating was done by incubating samples in an incubator at 40°C for 0 (control), 1, 2, 3, 4 and 5 days. After heating, the samples were transferred to germinate in a constant temperature room of 26°C , and light of 200 lux from fluorescent lamps for 15 hours per day. The percentage germination on the 10th day was analyzed with 4 or 5 replicates for each sample.

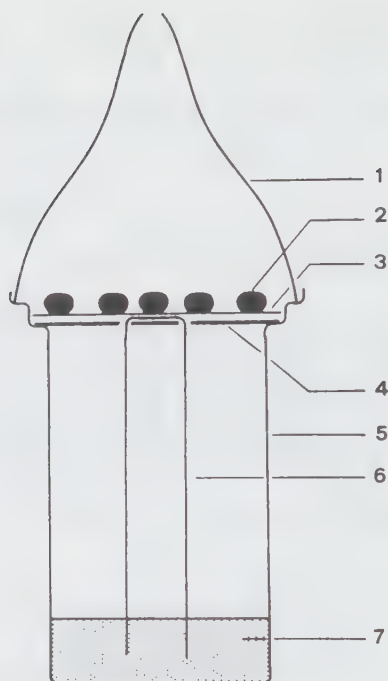


Fig. 4: The germination apparatus (no. 985/90, Semadeni AG, Ostermundigen). Seeds (2) on two layers of filter-paper (3) are constantly kept moist by using a filter-paper wick (6). The vessel (5) contains 60 ml distilled water (7). The plate (4) is used for supporting the filter-papers and an aerated cover (1) of a transparent material for covering the seeds.

2.9. Seed storage and longevity

2.9.1. Determination of seed moisture content

Clean seeds with an initial percentage germination of at least 90% were used as experimental material. Seed moisture content on wet weight basis was determined by a two stage drying method at 105°C as following:

The first drying stage was carried out by heating the seeds in the dishes in an incubator at $105 \pm 1^{\circ}\text{C}$ for 12 hours. After termination of the drying period, the dishes were immediately covered and placed in a desiccator to cool for about 30 minutes, then weighed. The moisture lost by the first drying stage calculated on wet basis and expressed in percentage, is:

$(M_2 - M_3)100 / (M_2 - M_1)$, where M_1 is the weight in grammes of the dish and its cover, M_2 is the weight in grammes of the dish, its cover and its contents and M_3 is the weight in grammes of the dish, its cover and contents after drying. The second drying stage was done and calculated as in the first drying stage. Each sample was carried out with 5 replicates.

2.9.2. Determination of percentage viability

In this experiment the percentage germination of samples was adopted as the percentage viability, determined by sowing seeds from different periods of storage in 3 combinations of seed moisture content and constant storage temperature. Variations of seed moisture content were prepared by drying seeds in a desiccator for 1-3 days. Three combinations of moisture content and constant storage temperature are as shown below (Table 2).

Samples consisting of 30 or 50 seeds with 4 or 5 replicates were sown in petri dishes, diameter 9 cm, on 2 layers filter-paper. Before sowing scarification should be done for hardness of seed coat. Germination was carried out for 20 days in a room of 26°C and lighted with fluorescent lamps for 15 hours per day (500 lux). Percentage germination on the 20th day was adopted as percentage viability.

Table 2: The combination of seed moisture content and constant storage temperature

Species	Moisture content (%)	Temperature * (°C)
<u>Amaranthus tricolor</u>	1. 11	50
	2. 13	50
	3. 13	60
<u>Cajanus cajan</u>	1. 12	40
	2. 14	40
	3. 12	60
<u>Leucaena leucocephala</u>	1. 4	50
	2. 6	50
	3. 6	60
<u>Phaseolus aureus</u>	1. 8	40
	2. 13	47
	3. 13	60
<u>Phaseolus pubescens</u>	1. 15	40
	2. 17	40
	3. 15	60
<u>Sesamum orientale</u>	1. 5	40
	2. 9	47
	3. 7	60
<u>Sesbania bispinosa</u>	1. 12	40
	2. 12	58
	3. 14	60
<u>Vigna unguiculata</u> var. <u>cylindrica</u>	1. 11	47
	2. 14	47
	3. 14	60
<u>Vigna unguiculata</u> var. <u>sinensis</u>	1. 11	40
	2. 6	60
	3. 11	60

* Temperature fluctuation of incubators $\pm 1^{\circ}\text{C}$, seeds stored in sealed vials.

2.9.3. Determination of regeneration intervals of seed

These experiments were designed to estimate regeneration intervals by applying the three viability equations and the four viability constants determined at higher temperature to predict the time for viability to fall by 5% at -6°C with moisture content 5%.

As pointed out by Roberts (1972) "Seed survival curves under constant environmental conditions are negative cumulative normal distribution". "The distribution of death for the whole population can thus be expressed as:

$$Y = \frac{1}{\sigma \sqrt{2\pi}} e^{\frac{-(p - \bar{p})^2}{2\sigma^2}}$$

where Y is the relative frequency of deaths occurring at time p, $\bar{p}$ is the mean viability period and σ is the standard deviation of the distribution of deaths in time."

"The spread of the distribution in time is proportional to the mean viability period. This has been expressed (Roberts, 1960, 1961; Roberts and Abdalla, 1968) as: $\sigma = K_{\sigma} \cdot \bar{p}$ where, K_{σ} is a constant for the species." The relationship between moisture content, temperature and mean viability period is described by the equation $\log \bar{p} = K_v - c_1 m - c_2 t$ where m is moisture content (% fresh weight basis), t is temperature ($^{\circ}\text{C}$) and K_v , c_1 and c_2 are constant (Roberts, 1972; Roberts & Ellis, 1977).

For the analysis of the experimental results, probit-analysis by linear regression with the maximum-likelihood method (Weber, 1972) was used. The analysis was programmed with FORTRAN in a computer by using an IBM 2741 Communication Terminal.

The following table 3 shows how the experimental data are prepared and then analyzed (a to f).

- a) no. 4. Table 28 (Weber, 1972) with value Y
 no. 5. Probit with 2 decimals
 no. 6. Table 29 (Weber, 1972) with value Y0
 no. 8. $Y1 = (Y0 - P/Z) + p/Z$ where: $(Y0 - P/Z)$
 and $1/Z$ from Table 29 and $p = Y/100$
- b) Calculation of $\bar{X}$ and $\bar{Y}_1$
 $\bar{X} = S2/S1$ and $\bar{Y}_1 = S3/S1$
- c) Calculation of coefficient and equation of regression: $S6 = S4 - (S2)^2/S1$, $S7 = S5 - (S2)(S3)/S1$
 $b = S7/S6$ and $y = \bar{Y}_1 + b(x - \bar{X})$
- d) Calculation of mean of viability period ($\bar{p}$) and standard deviation (σ). By $y = 5$ for $y = \bar{Y}_1 + b(x - \bar{X})$, so $x = \bar{p} \cdot MS(\bar{p}) = 1/b^2 (1/S1 + (\bar{p} - \bar{X})^2/S6)$
 $\sigma = \sqrt{MS(\bar{p})}$
- e) Calculation: the constants of viability (K_σ , K_v , c_1 and c_2)
 $K_\sigma = \sigma/\bar{p}$; with 3 combinations of temperature and seed moisture content, 3 values of $\bar{p}$ can be obtained and by equation $\log \bar{p} = K_v - c_1 m - c_2 t$, K_v , c_1 and c_2 can be obtained.
- f) The mean viability period $\bar{p}$ for storage at -6°C and moisture content of 5% can be calculated with the equation $\log \bar{p} = K_v - c_1 m - c_2 t$. By solving the equation $p_{95} = \bar{p} - 1.645 K_\sigma \bar{p}$, the probable regeneration interval (p_{95}) for storage condition -6°C moisture content of 5% and viability to fall by 5% can be obtained.

2.10. Effect of storage conditions on germination ability

2.10.1. Effect of storage treatment on cardinal temperatures of germination

Amaranthus paniculatus seeds with moisture content of 7% were stored at 65°C for 106 days. Seeds before and after this storage treatment were sown in compartments of the thermogradient bar apparatus (see 2.3.). Each compartment contained 50 seeds. The effect of storage temperature on cardinal temperatures of germination is shown by plotting the successive days for 50% germination against temperature.

2.10.2. Effect of prestorage treatment on cardinal temperatures of germination

Seeds of Capsicum annum with a moisture content of 9% were stored for 177 days at 40°C with prestorage at 50°C for 3, 4 and 5 days. After storage the seeds were then germinated in the thermogradient bar apparatus, each compartment contained 10 seeds.

2.10.3. Effect of storage treatment on chilling sensitivity

Seeds of Amaranthus paniculatus with moisture contents of 7%, before and after being kept at 65°C for 106 days were used for this experiment. Both of these seed treatments were chilled at various temperatures from 2°C to 10°C for 15 days in compartments of the thermogradient bar apparatus in moist conditions. Then they were transferred to germinate in petri dishes, diameter 9 cm on 2 layers of filter-paper, in a room at 26°C for 10 days with light of 500 lux from fluorescent lamps for 15 hours per day.

2.10.4. Effect of prestorage treatment on chilling sensitivity

The Capsicum annum seeds which had been stored at 40°C for 177 days with prestorage at 50°C for 3, 4 and 5 days were used for experiment. Ten seeds in each compartment of thermogradient bar apparatus were chilled and germinated as in experiment 2.7.

2.10.5. Effect of storage on heat sensitivity

The Amaranthus paniculatus seeds before and after storage at 65°C for 106 days were used for this experiment. In each treatment 4 replicates, each of 50 seeds were heated and germinated as in experiment 2.8.

3. RESULTS AND DISCUSSION

3.1. Temperature span of germination

Twenty six species collected from various places in Java, India and Switzerland were compared. Their temperature characteristics allows to differentiate roughly three groups. A wide temperature span of germination was shown by the seed samples of the following species: Sesamum orientale, Amaranthus paniculatus, Sesbania bispinosa and Phaseolus aureus. A medium span was shown by Amaranthus spinosus, Amaranthus tricolor, Ocimum americanum, Albizia lophantha, Leucaena leucocephala, Cassia tora, Taraxacum officinale, Elaeagnus conferta, Ageratum conyzoides, Talinum paniculatum, Celosia argentea f. plumosa. The rest of the species had a narrow temperature span of germination. Table 4 summarizes the cardinal temperatures of germination, while figures 5 and 6 show different types of temperature span for germination. It can be seen clearly that the temperature span for the five *Amaranthaceae* species investigated is wider than for the two *Lamiaceae* and the two *Rubiaceae* species. The width of the temperature span is nearly equal for the *Amaranthaceae* and the *Papilionaceae* species, while that for the two *Mimosaceae* is definitely narrower. It can also be seen that the variation of the temperature span is mostly due to the variation of the maximum temperature of germination.

By plotting temperature span against the temperature axis, the distribution of thermal germination abilities of Javanese species is studied. The seedling establishment capacity in the different thermal regions can thus be identified. The relative number in percent of species germinating at any temperature is taken as "index of germination ability". As 21 species have been investigated, this

Table 4: The cardinal temperatures of germination

No. Species	Temperature ($^{\circ}\text{C}$)*		
	Minimum	Optimum	Maximum
1. <u>Ageratum conyzoides</u>	13(19)	19-23(9)	26(10)
2. <u>Albizia lophantha</u>	10(11)	19(5)	27(9)
3. <u>Amaranthus caudatus</u>	11(9)	26-34(1)	41(9)
4. <u>Amaranthus paniculatus</u>	12(9)	25-29(3)	35(6)
5. <u>Amaranthus spinosus</u>	17(6)	29(1)	36(4)
6. <u>Amaranthus tricolor</u>	14(12)	21-31(2)	34(5)
7. <u>Borreria brachystema</u>	20(10)	21-25(10)	25(10)
8. <u>Cassia tora</u>	15(16)	25-29(4)	33(14)
9. <u>Celosia argentea f. plumosa</u>	16(7)	21-35(2)	35(3)
10. <u>Cleome gynandra</u>	28(7)	28-32(7)	33(7)
11. <u>Crotalaria mucronata</u>	13(15)	34(5)	34(5)
12. <u>Elaeagnus conforta</u>	14(10)	19-29(5)	33(6)
13. <u>Hypericum leschenaultii</u>	14(15)	18-20(2)	21(12)
14. <u>Hyptis brevipes</u>	15(16)	21-23(6)	27(15)
15. <u>Impatiens javensis</u>	17(8)	24(7)	24(7)
16. <u>Leucaena leucocephala</u>	14(22)	21-23(16)	30(17)
17. <u>Mycetia cauliflora</u>	18(18)	22(9)	24(11)
18. <u>Myrsine affinis</u>	18(13)	19(12)	22(21)
19. <u>Ocimum americanum</u>	13(11)	17-25(2)	28(5)
20. <u>Phaseolus aureus</u>	6(10)	20-32(3)	34(3)
21. <u>Phaseolus pubescens</u>	14(9)	21-31(3)	35(6)
22. <u>Polygonum chinense</u>	10(20)	19(9)	21(12)
23. <u>Sesamum orientale</u>	15(9)	26-34(2)	37(3)
24. <u>Sesbania bispinosa</u>	13(11)	32(2)	38(5)
25. <u>Talinum paniculatum</u>	15(19)	22-30(6)	30(6)
26. <u>Taraxacum officinale</u>	8(10)	22(2)	29(6)

* The numbers out and inside parentheses are temperature and day of incubation at which germination of 50% is achieved respectively.

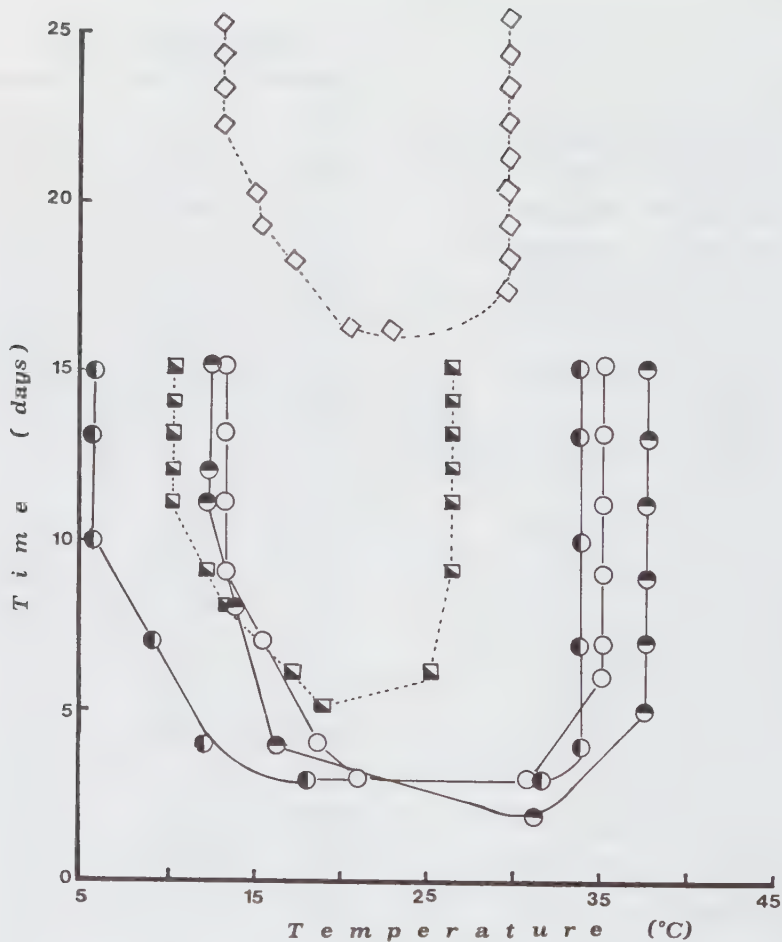


Fig. 5: Germination curves of species from the families Mimosaceae (*Albizia lophantha* ■ , *Leucaena leucocephala* ◇) and Papilionaceae (*Phaseolus aureus* ○ , *Phaseolus pubescens* ● , *Sesbania bispinosa* ●). Successive days for 50% germination were plotted against temperature.

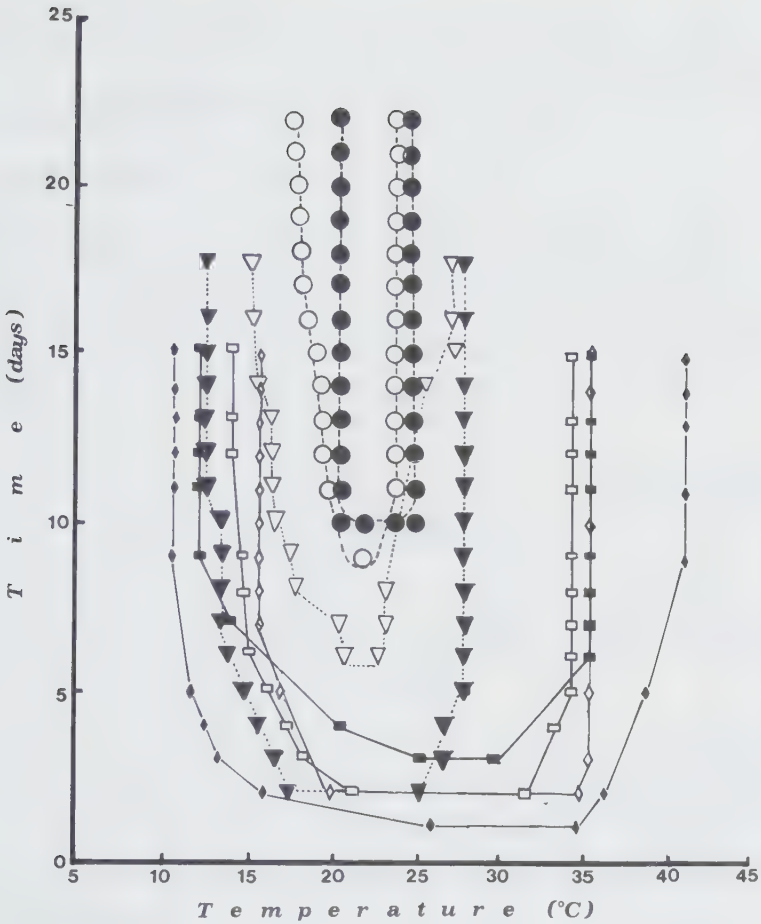


Fig. 6: Germination curves of species from the families Amaranthaceae (*Amaranthus spinosus* *, *A. caudatus* ♦, *A. paniculatus* ■, *A. tricolor* □, *Celosia argentea* f. *plumosa* ◇), Lamiaceae (*Ocimum americanum* ▼, *Hypstis brevipes* ▽), and Rubiaceae (*Borreria brachystema* ●, *Mycetia cauliflora* ○).

Successive days for 50% germination were plotted against temperature of germination.

* See Fig. 15.

index is $(y/21) \cdot 100\%$, where y is the number of germinating species at one thermal region (Fig. 7). At a temperature of $20-21^{\circ}\text{C}$, 95% of the species investigated are able to germinate. The frequency distribution shows clearly a prevalence for higher temperatures in our species sample. In Java (Braak, 1945) as in other countries with a moist tropical climate altitude is the most important factor influencing the temperature regime. The average temperature is even in the mountains rather constant, gradually and regularly decreasing with altitude, with regular daily, but not seasonal variation (Backer & Brink, 1965). It is suggested that the effect of higher

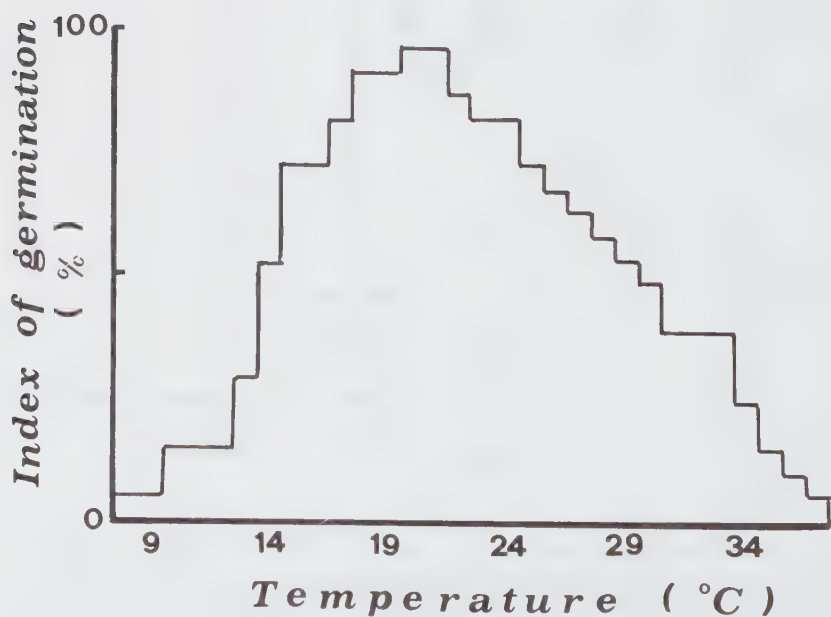


Fig. 7: Thermal distribution of germination ability of 21 species collected from Java. The temperature span of germination is plotted along the temperature-axis. The index of germination ability in a thermal region is expressed by $(y/21) \cdot 100\%$, y is the number of germinating species at that thermal region, 21 is the total number of species investigated.

altitude on reproduction is due to temperature. A good illustration of this influence is shown by Machaerina rubiginosa, Eleocharis dulcis and Scirpus juncus. These three species are common in the lowland of Java, they occur in higher altitudes however only in sterile state, propagating there vegetatively.

Temperatures with an index of germination ability of higher than 50% may be considered a favourable thermal region for seedling establishment. Based on this assumption 4 species groups can be identified, i.e.:

- A. Species germinating well from 14 to 29°C: Hyptis brevipes, Borreria brachystema, Mycetia cauliflora, Hypericum leschenaultii, Impatiens javensis, Myrsine affinis.
- B. Species germinating from 29°C to lower than 14°C: Ocimum americanum, Albizia lophantha, Polygonum chinense, Taraxacum officinale, Ageratum conyzoides.
- C. Species germinating from 14 to higher than 29°C: Amaranthus spinosus, Amaranthus tricolor, Celosia argentea f. plumosa, Leucaena leucocephala, Phaseolus pubescens, Sesamum orientale, Cassia tora, Cleome gynandra, Elaeagnus conferta, Talinum paniculatum.
- D. Species germinating from lower than 14°C to higher than 29°C: Crotalaria mucronata, Amaranthus caudatus, Amaranthus paniculatus, Sesbania bispinosa, Phaseolus aureus.

Based on width and position of temperature span, the samples from Switzerland and India belong to group D (Amaranthus caudatus, Amaranthus paniculatus, Phaseolus aureus, and Sesbania bispinosa, germinating from lower than 14°C to higher than 29°C) and group C (Celosia argentea, germinating from 14°C to 29°C).

Outstanding for the wide span of germination are the species of genera Amaranthus and Crotalaria. It is plausible that this germination characteristic has contributed to the wide geographical range of the species. They are found in different altitude of all tropical regions as weeds, crops or miscellaneous adventives. Most species of the genus Crotalaria occur in both parts of Old World (Good, 1964). The genera Hypericum, Polygonum, Impatiens, Taraxacum as well as Amaranthus are cosmopolitan and typical of temperate climates. In Java species of the first four genera are mostly found in high altitudes, it seems that this distribution characteristics associates with their ability to germinate also in warmer conditions. Albizia lophantha able to germinate well at relatively low temperatures is common in Java at higher altitudes and is found in lower altitudes in sterile state and propagating vegetatively. It is astonishing that nothing is known about the physiological basis of this phenomenon.

3.2. Cardinal temperatures of germination

The cardinal temperatures of germination are minima, maxima and optima. A wide variation in temperature minima for different crop plants has been reported; it has been suggested that the speed of germination at low temperatures and the position of temperature minima for germination, may be correlated with the geographical distribution of different species of Caryophyllaceae (Thompson, 1968). In the previous sub-chapter it was reported that species of group B can germinate at lower than 14°C; a comparison of the members (Table 4) shows that Taraxacum officinale, Albizia lophantha and Polygonum chinense have lower temperature minima (8° - 10°C) than Ageratum conyzoides and Ocimum americanum.

Temperature minima and geographical or ecological distribution appeared to be correlated with Albizia lophantha, Polygonum chinense, and Taraxacum officinale. They occur mainly in the mountains of the wet regions in Java (Table 15) up to altitudes of 2800 m (Polygonum chinense) or more (Albizia lophantha 3300 m, Taraxacum officinale 3000 m), while Ageratum conyzoides which shows a temperature minimum of 13°C occupies mainly lowland of drier regions, it can sometimes be found in altitudes up to 2100 m. The germination lag of these three species (10-20 days) was shorter than for Ageratum conyzoides (29 days). It is suggested that species with lower temperature minima germinate more effectively in low temperature of any thermal region than species with higher temperature minima. A similar phenomenon has been shown in some species of Caryophyllaceae (Thompson, 1970). Ocimum americanum is found in altitudes of 1-300 m (Backer & Brink, 1965) and its temperature minimum is 13°C , this corresponds with the general relation although the species is mostly cultivated.

Amaranthus caudatus, Amaranthus paniculatus, Sesbania bispinosa and Phaseolus aureus are cultivated plants and the seed samples were collected from India and Switzerland. Their ability to germinate at temperatures lower than 14°C may have been selected during the cultivation in temperate regions.

The temperature maxima for germination are broadly similar in the species Cassia tora, Cleome gynandra, Elaeagnus conferta and Crotalaria mucronata ($33-34^{\circ}\text{C}$). Extremely high ($35-37^{\circ}\text{C}$) is the maximum in Amaranthus spinosus, Sesamum orientale, and Phaseolus pubescens. These three species are sometimes found in altitudes of up to 1400 m, but grow well only in the lowland of drier regions as cultivated plants (Sesamum orientale, Phaseolus pubescens) or weed (Amaranthus spinosus).

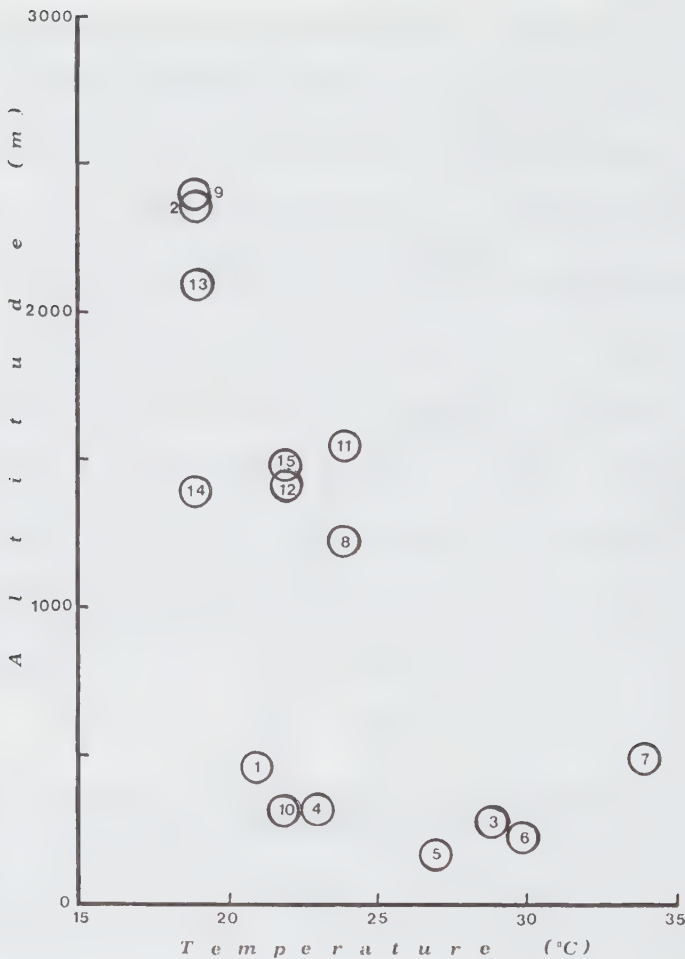


Fig. 8: Relation between mean altitude of distribution and optimum temperature of germination of non cultivated species in Java. (Mean altitude of distribution is plotted against optimum temperature of germination).

Numbers correspond to the following species:

- | | |
|--------------------------------|-----------------------------------|
| 1. <u>Ageratum conyzoides</u> | 9. <u>Hypericum leschenaultii</u> |
| 2. <u>Albizia lophantha</u> | 10. <u>Hyptis brevipes</u> |
| 3. <u>Amaranthus spinosus</u> | 11. <u>Impatiens javensis</u> |
| 4. <u>Borreria brachystema</u> | 12. <u>Mycetia cauliflora</u> |
| 5. <u>Cassia tora</u> | 13. <u>Myrsine affinis</u> |
| 6. <u>Cleome gynandra</u> | 14. <u>Polygonum chinense</u> |
| 7. <u>Crotalaria mucronata</u> | 15. <u>Taraxacum officinale</u> |
| 8. <u>Elaeagnus conforta</u> | |

The temperature optimum varies widely from 18° to 35°C . Low temperature optima ($18\text{-}20^{\circ}\text{C}$) are shown by species which occur only in high altitudes. To this group belong Albizia lophantha, Hypericum leschenaultii and Myrsine affinis, with the exception of Polygonum chinense which is found also in lowland. Some species have a broad range of optimum temperature for germination, for example: Ocimum americanum ($17^{\circ}\text{-}25^{\circ}\text{C}$), Amaranthus tricolor ($21^{\circ}\text{-}31^{\circ}\text{C}$), Celosia argentea f. plumosa ($21^{\circ}\text{-}35^{\circ}\text{C}$), Sesamum orientale ($26^{\circ}\text{-}34^{\circ}\text{C}$), Phaseolus aureus ($20\text{-}32^{\circ}\text{C}$) etc. This property may be characteristic for species growing well in a wide geographical or altitudinal areas. By plotting the mean altitude of distribution of non cultivated species against the temperature optimum (see Table 4 and 15), a correlation between the two parameters can be shown. The mean altitude was obtained from all altitudes recorded on herbarium material from Herbarium Bogoriense (Indonesia) and the literature. A significant correlation between optimum temperature of germination and mean altitude at 99% level, r (correlation coefficient) = -0.67 could be calculated. It is suggested that the temperature optimum is useful for control of altitudinal distribution of species in Java.

3.3. Effects of constant temperature on germination

At constant temperatures, germination rates increase with higher temperature. In Taraxacum officinale the lowest temperature for germination is 5°C and in Ageratum conyzoides 11°C (Fig. 9). The minimum temperature at which 50% germination is reached by Taraxacum officinale from Tawangmangu (Central Java) at 8°C , from Valle (Airolo, Switzerland) at 9°C and by Ageratum conyzoides

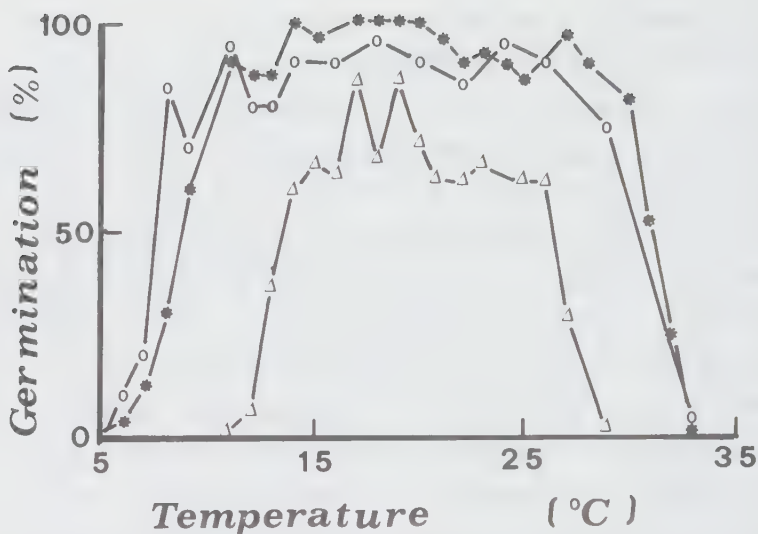


Fig. 9: Percentage germination on successive days after sowing on a thermogradient bar for 25 days of Taraxacum officinale from Tawangmangu (o), from Valle, Tessin (*) and Ageratum conyzoides from Jogjakarta (Δ)

from Jogjakarta (Central Java) at 13°C (Fig. 10). Speed and rate of germination are also faster and higher with increasing temperature until the optimum temperature – which is in Taraxacum officinale from Tawangmangu and Valle 22°C and 20°C respectively, in Ageratum conyzoides 19–23°C. At still higher temperatures rate and speed of germination decrease again. We may explain that higher temperatures promotes the reaction rates. Weak seed which can not synchronise their biochemical processes die. Those seeds which can maintain these faster reaction rates do germinate faster. At higher temperatures than the optimum, the metabolic imbalance becomes so severe that the ability of germination is also reduced and shows a progressive decline.

Most species germinate slower at the temperature minima than at the optima. There is nearly always a small difference between the

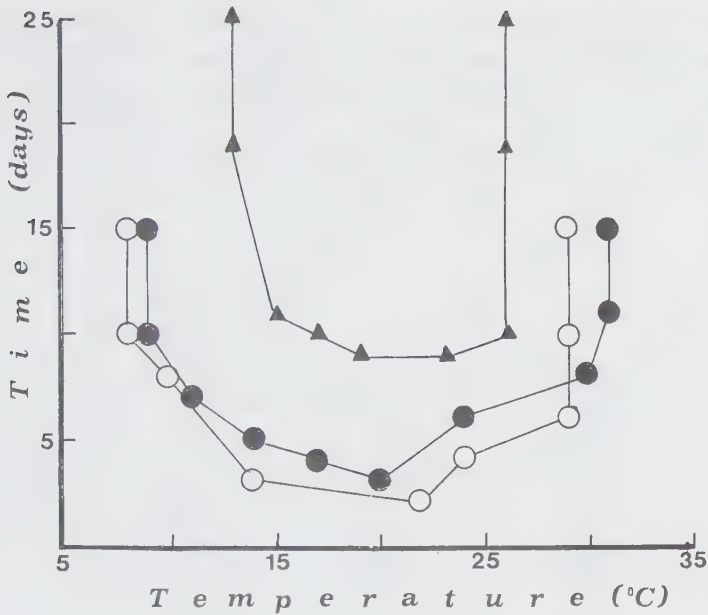
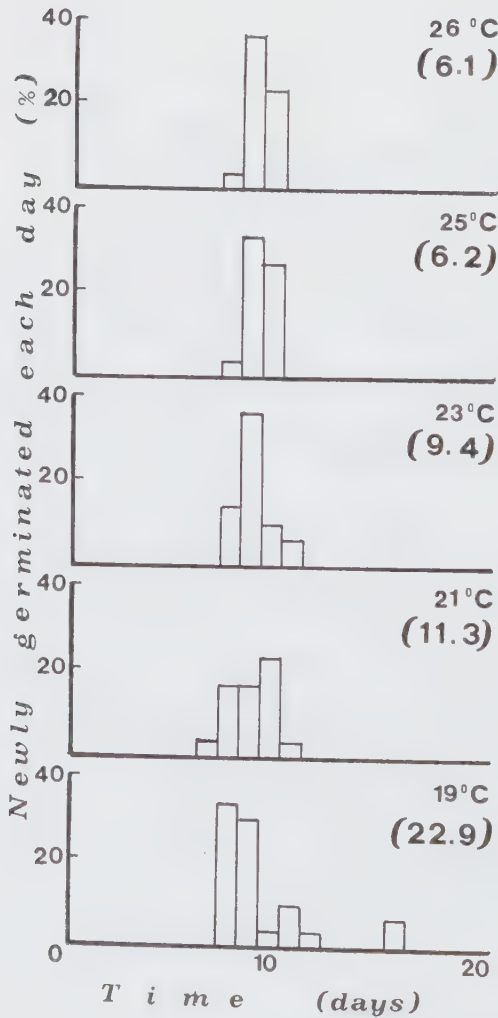


Fig. 10: Germination curves of a population of *Taraxacum officinale* from Tawangmangu O, from Valle, Tessin ● and *Ageratum conyzoides* from Jogjakarta ▲.

speed of germination at temperature maxima and optima. This germination behavior at high temperatures maybe significant from an agronomic as well as a physiological viewpoint, for instance for producing large numbers of seedlings quickly at high temperatures. It has been reported that individuals selected out by high temperature show an increase in 'vigor' over the basic population (Gulliver & Heydecker, 1973), therefore this process could provide a useful basis for a breeding programme. Temperature influences not only speed and percentage of germination, but can also affect the uniformity of germination. At certain low temperatures, the rate of germination is very low, but the spread of germination in time is great. The distribution of germination in time of *Ageratum conyzoides* shows clearly a greater uniformity at the higher tempera-

ture of germination (Fig. 11). The uniformity of germination has been investigated in a temperature range from 26°C (maximum temperature) to 13°C (minimum temperature), by calculating its coefficient of variation ($CV = s(100)/m$; s = standard deviation, m = mean).



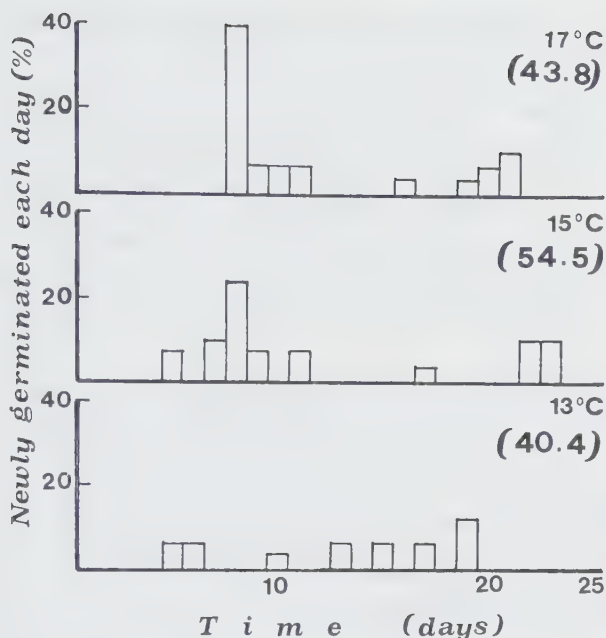


Fig. 11: Germination distribution in time of *Ageratum conyzoides* at different temperatures. Coefficient of variation of the distribution of germination in time (number in parenthesis).

The coefficients of variation (6.1 to 11.3) for germination at 26° and 21°C, differ only slightly. In contrast to germination at lower temperatures, from 21° to 15°C, the coefficient of variation increases significantly from 11.3 to 54.5, with germination at 13°C the coefficient of variation is still 40.4.

This relationship between temperature of germination and coefficient of variation of the germination distribution in time is important in ecological and physiological respects. By changing the uniformity of the germination distribution in time, species can regulate their seedling establishment. At low temperature a great distribution of germination in time is more successful. Seedlings are then produced continually during a long period of time.

3.4. Germination at fluctuating temperature

Diurnal fluctuations of temperature may also initiate or accelerate germination in certain flowering plants (Morinaga, 1926; Strafford, 1965; Frecht et al, 1973; Thompson, 1974a, 1974b). The effectiveness of the response varies according to the amplitude of fluctuation and the presence or absence of light (Thompson, 1974b; Thompson et al, 1977).

The ecological significance of this response to a diurnal alternation of temperature may be that it promotes the germination of those seeds which are close to the soil surface, where such temperature fluctuation occurs. Why a fluctuating temperature should stimulate seeds to germinate is not fully understood, but it has been suggested that alternating temperatures may increase the permeability of membranes and seed coats. An other possibility is that an increase in temperature above the average causes a temporary increase in concentration of some substance(s) to a critical level which permits or conditions germination. Cohen (1958) concluded that increasing the temperature to a critical level destroys an inhibitor.

Very little is known about the influence of fluctuating temperature on the germination of tropical plants, therefore fourteen species were chosen for a corresponding experiment, the results are summarized in Table 5. Germination of Cassia tora, Taraxacum officinale and Ageratum conyzoides in darkness either at constant or alternating temperatures failed to achieve 50% germination. Cleome gynandra and Amaranthus spinosus germinated under darkness at constant temperatures of 10⁰, 17⁰, 22⁰ and 30⁰C to less than 50%, but by alternating temperatures of 22/30⁰ and 10/30⁰ reached a percentage of 50% even under darkness. Amaranthus spinosus is

Table 5: Germination (%) at constant and alternating temperature in darkness. The numbers are germination percent after ten days.

Species:	Temperature (°C)							
	10	10/17	17	17/22	22	22/30	30	10/30
<u>Ageratum conyzoides</u>	0	0.8	0	0	0	0	0	0
<u>Amaranthus paniculatus</u>	0.8	98.4	100	98.8	100	100	100	100
<u>Amaranthus spinosus</u>	0	0	24.2	30.8	24.2	56.7*	25.0	68.3*
<u>Amaranthus tricolor</u>	0	0	64.4	82.4	93.2	94.0	87.6	91.2
<u>Cassia tora</u>	0	20.0	43.8	45.0	43.8	28.8	37.5	46.3
<u>Cleome gynandra</u>	0	15.9	36.7	30.9	30.8	75.0	49.2	62.5
<u>Leucaena leucocephala</u>	0	15.2	29.2	10.4	50.8	24.8	44.5	57.6
<u>Phaseolus aureus</u>	50.8	60.0	74.2	90.0	80.0	90.8	97.5	84.2
<u>Phaseolus pubescens</u>	0	21.7	72.5	67.5	84.2	79.2	87.5	54.2
<u>Sesamum orientale</u>	0	0	12.0	70.0	82.8	80.4	82.8	72.0
<u>Sesbania bispinosa</u>	1.7	26.7	45.0	53.3	59.1	55.0	55.8	58.3
<u>Talinum paniculatum</u>	0	2.5	10.0	13.3	10.8	65.0	96.7	81.7
<u>Taraxacum officinale</u>	2.0	10.0	15.3	12.0	2.0	2.6	0	8.0
<u>Vigna unguiculata</u>	0	95.9	94.2	95.9	91.7	99.2	95.9	98.4

* Statistically significant (99% level) difference to germination rate at the constant temperatures.

a species showing well a positive influence of alternating temperature; germination at $22/30^{\circ}\text{C}$ (56.7%) was significantly better (at 99% level) than at 22°C (24.2%) and at 30°C (25°C), and also at $10/30^{\circ}\text{C}$ (68.3%) it was significantly higher (at 99% level) than at

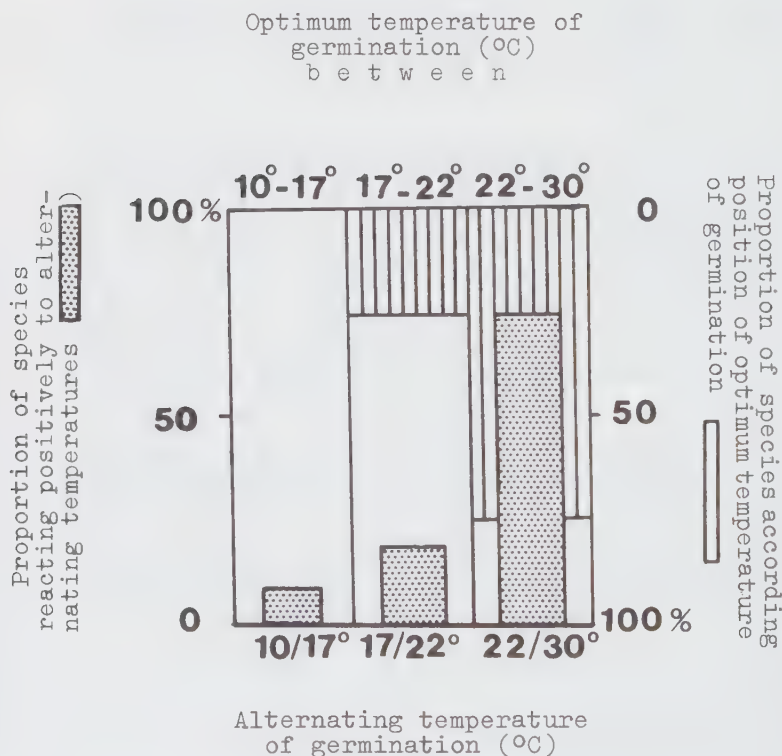


Fig. 12: Relationship between position of optimum temperature of germination and effectiveness of alternating temperature for germination.

Twelve species were investigated for their optimum temperatures of germination and their effectiveness to germinate at alternating temperatures. The highest percentage germination at $10/17^{\circ}$, $17/22^{\circ}$ and $22/30^{\circ}\text{C}$ was taken as a criterion of effectiveness. Positions of optimum temperature of germination were subdivided into 3 groups, i.e. between $10-17^{\circ}$, $17-22^{\circ}$ and $22-30^{\circ}\text{C}$.

30°C (25%) and at 10°C (0%). Most species germinated well when submitted to an alternating temperature of 22/30°C. It has been demonstrated, that the geographical distribution of some plants may be determined by the diurnal fluctuation of temperature during the growing season (Went, 1957). Fig. 12 shows that the closer the alternating temperature to the optimum temperature of germination, the more effective was the treatment. It suggest that the difference of the median fluctuating temperature from the optimum temperature of germination is important for promotion of germination at diurnal fluctuations of temperature. Diurnal fluctuations of temperature near the optimum may increase rate, speed and uniformity of germination.

3.5. Pre-imbibition

Seeds which are located on or very near the surface of the soil would be exposed to alternating temperature and daily period of light. Fluctuating temperature could be caused by water evaporation, wind or alternating illumination.

Very little is known about the influence of the early period of imbibition (pre-imbibition) on germination at various temperatures.

Woodstock and Pollock (1965) conclude that the early period of imbibition is critical, because at this time respiration has to be rapid enough to supply sufficient energy for the orderly rehydration and stretching of the membranes within the embryonic axis. Anaerobic conditions at these critical sites lead to irreversible membrane damage and hence to impairment of growth. Pollock and Toole (1966) found that provided the germination temperature

is high enough at the very start, lima bean seedlings become subsequently much more resistant to chilling. Protein and RNA synthesis also begin during this lag phase, and most probably are concerned initially with the synthesis of enzymes for the mobilization of other substances in the preparation of the tissues for germination. It has been shown that certain enzymes are absent from the mitochondria of dry seeds, and therefore these missing enzymes must be synthesized during early germination (Villiers, 1975).

In this experiment the influence of pre-imbibition on germination at constant and alternating temperatures has been studied.

Percentage germination either at constant or alternating temperature (Table 6 and 7) were not influenced by pre-imbibition under light for 5 hours at a temperature of 26°C in the following species: Amaranthus spinosus, Amaranthus tricolor, Cassia tora, Phaseolus pubescens, Sesamum orientale, Sesbania bispinosa and Vigna unguiculata var. cylindrica. Percentage germination of Cassia tora in darkness was lower than 50%. It could be shown that the seeds needed light for germination (Table 9). Darkness is sufficient for germination for the other species.

This pre-imbibition treatment promoted however significantly (at 99% level) germination of Ageratum conyzoides at constant temperature 17°C and at alternating temperatures of $17/22^{\circ}$ and $22/30^{\circ}\text{C}$. Light is needed for germination. During pre-imbibition light is more effective for promotion of germination at alternating temperature than at constant temperature. Promotion of germination by pre-imbibition under light was also significant (at 99% level) in Talinum paniculatum at 17°C , $17/22^{\circ}\text{C}$ and 22°C . During pre-imbibition light is absolutely needed (Table 8) for promotion of germination. However for germination without pre-imbibition at high

Table 6: Germination (%) in darkness at constant temperatures after pre-imbibition in light at 26°C for 0 and 5 hours.

Species	Hours imbi- bition	Temperature (°C)			
		10	17	22	30
1. <u>Ageratum conyzoides</u>	0	0	0*	0	0
	5	0	15.0	0	0
2. <u>Amaranthus paniculatus</u>	0	0.8*	100	100	100
	5	44.0	99.6	99.6	100
3. <u>Amaranthus spinosus</u>	0	0	24.2	24.2	25.0
	5	0	35.8	40.0	44.2
4. <u>Amaranthus tricolor</u>	0	0	64.4	93.2	87.6
	5	0	71.6	92.4	97.6
5. <u>Cassia tora</u>	0	0	43.8	43.8	37.5
	5	0	41.3	47.5	35.0
6. <u>Cleome gynandra</u>	0	0	36.7	30.8	49.2
	5	0	32.5	28.3	30.8
7. <u>Leucaena leucocephala</u>	0	0	29.2**	50.8	44.5
	5	0	8.0	34.8	39.2
8. <u>Phaseolus aureus</u>	0	50.8*	74.2	80.0	97.5
	5	70.0	86.7	84.2	97.5
9. <u>Phaseolus pubescens</u>	0	0	72.5	84.2	87.5
	5	0	70.0	80.0	91.7
10. <u>Sesamum orientale</u>	0	0	12.0	82.8	82.8
	5	0	3.6	75.6	78.0
11. <u>Sesbania bispinosa</u>	0	1.7	45.0	59.1	55.8
	5	6.6	55.0	60.0	63.3
12. <u>Talinum paniculatum</u>	0	0	10.0*	10.8*	96.7
	5	0	95.9	95.0	99.2
13. <u>Taraxacum officinale</u>	0	2.0	15.3*	2.0*	0*
	5	6.0	54.7	35.3	12.7
14. <u>Vigna unguiculata</u>	0	0	94.2	91.7	95.9
<u>var. cylindrica</u>	5	0	99.2	99.2	98.4

* Lower significantly (99% level) than after pre-imbibition for 5 hours.

** Higher significantly (99% level) than after pre-imbibition for 5 hours.

Table 7: Germination (%) in darkness at alternating temperatures after pre-imbibition in light at 26°C for 0 and 5 hours.

Species	Hours imbi- bition	Temperature (°C)			
		10/17	17/22	22/30	10/30
1. <u>Ageratum conyzoides</u>	0	0.8	0*	0*	0
	5	5.0	15.0	15.0	3.3
2. <u>Amaranthus paniculatus</u>	0	98.4	98.8	100	100
	5	99.6	100	99.6	100
3. <u>Amaranthus spinosus</u>	0	0	30.8	56.7	68.3
	5	3.3	44.2	74.2	80.9
4. <u>Amaranthus tricolor</u>	0	0	82.4	94.0	91.2
	5	0	86.8	94.0	93.2
5. <u>Cassia tora</u>	0	20.0	45.0	28.8	46.3
	5	17.5	43.8	26.3	37.5
6. <u>Cleome gynandra</u>	0	15.9	30.9	75.0**	62.5
	5	5.0	20.8	42.5	54.2
7. <u>Leucaena leucocephala</u>	0	15.2	10.4	24.8	57.6
	5	22.4	10.4	34.8	50.8
8. <u>Phaseolus aureus</u>	0	60.0	90.0	90.8	84.2
	5	65.0	89.2	93.3	89.2
9. <u>Phaseolus pubescens</u>	0	21.7	67.5	79.2	54.2
	5	25.9	65.9	76.7	60.0
10. <u>Sesamum orientale</u>	0	0	70.0	80.4	72.0
	5	0.4	73.2	79.2	75.6
11. <u>Sesbania bispinosa</u>	0	26.6	53.3	55.0	58.3
	5	34.1	52.5	52.5	62.5
12. <u>Talinum paniculatum</u>	0	2.5	13.3*	65.0	81.7
	5	15.0	97.5	99.2	85.9
13. <u>Taraxacum officinale</u>	0	10.0*	12.0*	2.6*	8.0
	5	38.0	87.4	30.7	20.7
14. <u>Vigna unguiculata</u> <u>var. cylindrica</u>	0	95.9	95.9	99.2	98.4
	5	93.4	93.4	99.2	98.4

* Lower significantly (99% level) than after pre-imbibition for 5 hours.

** Higher significantly (99% level) than after pre-imbibition for 5 hours.

Table 8: Germination (%) without and with pre-imbibition treatment under light and darkness.

Species	Temperature of germination (°C)	Without pre-imbibition	Pre-imbibition at 26°C in:	
			darkness	light
<u>Amaranthus paniculatus</u>	10	0	65.85*	40.83*/***
<u>Talinum paniculatum</u>	17	16.65	8.35	94.18*/**
	17/22	9.15	3.33	95.00*/**
	22	50.85	53.33	100 */**

* Higher significantly (at 99% level) than percentage germination without pre-imbibition.

** Higher significantly (at 99% level) than percentage germination with pre-imbibition in darkness.

*** Lower significantly (at 99% level) than percentage germination with pre-imbibition in darkness.

Table 9: Percentage germination at constant temperatures in darkness and continuous light.

Species	Temperature (°C)	Percentage germination* (%)	
		darkness	continuous light
<u>Ageratum conyzoides</u>	10	0	0
	17	0	53.3
	22	0	63.3
	30	0	0
<u>Cassia tora</u>	10	0	0
	17	43.8	25.0
	22	43.8	55.0
	30	37.5	65.0
<u>Taraxacum officinale</u>	10	2.0	70.0
	17	15.3	95.0
	22	2.0	85.0
	30	0	75.0

* Germination for 10 days under darkness and continuous light of 50 lux from fluorescent lamps.

(30°C) temperature or alternating temperature of 10/30°C under darkness is favourable for germination. In Taraxacum officinale pre-imbibition promoted germination significantly (at 99% level) at temperatures: 10/17°, 17°, 17/22°, 22°, 22/30° and 30°C. It could be shown that the seeds needed light for germination (Table 9). Germination at 10°C of Amaranthus paniculatus and Phaseolus aureus was also significantly promoted by pre-imbibition treatment under light. Both species germinate well in darkness, even in Amaranthus paniculatus a light treatment during pre-imbibition is less effective than complete darkness for promotion of germination at a temperature of 10°C.

A pre-imbibition treatment was found to inhibit germination of Cleome gynandra at alternating temperature of 22/30°C and in Leucaena leucocephala at constant temperature of 17°C. Light during pre-imbibition inhibited germination of those species.

3.6. Response of chilling on germination

In table 10 the 25 species are classified into two groups, i.e.: non cultivated and cultivated species, for each of which the proportion of species responding to chilling pretreatments at various temperatures has been calculated. Response of chilling on germination of each species is presented in table 11.

In non cultivated species, chilling pretreatment at any temperature between 2-10°C stimulates or promotes germination better than in cultivated species. The highest chance for a promoted germination is obtained after chilling at a temperature of 6.1-8°C. In contrast, a chilling pretreatment with cultivated species gives a small chance

of promotion at any temperature between 2-10°C, germination may be inhibited by chilling pretreatment.

Table 10: Frequency of positive response by chilling pretreatment on the germination of non cultivated and cultivated species groups.

	No. of species investi- gated	% promotion of germination* after chilling at temperature (°C)			
		2.1-4.0	4.1-6.0	6.1-8.0	8.1-10
Non cultivated species	14	35.7	42.9	57.1	42.9
Cultivated species	11	9.1	9.1	9.1	9.1

* A positive effect of a chilling pretreatment is assumed when the germination rate is at least 5% higher than in the control.

The reasons for the failure of germination after chilling in cultivated species are unknown. However it is interesting to note that some tropical and subtropical plants are killed by chilling to temperatures still well above zero, whereas others, notably arctic-alpine plants, can survive even prolonged exposure to sub-zero temperatures. The other phenomenon which is known as acclimatization shows that the freezing resistance of most plants changes with the season in step with changes in environmental temperature (Sutcliffe, 1977). During the long period of cultivation a high speed and great uniformity of germination has been selected and maintained in crop population, in other words, dormancy was counter-selected.

It is known that seeds in a dormant state are more resistant to unfavourable conditions than seeds in a non dormant state. In some

species, percentage germination of seeds still can be increased or promoted by factors of relieving dormancy, for instance by chilling treatment.

Table 11: Chilling response on germination*

Cultivated species	Chilling temperature (°C)			
	2.1-4	4.1-6	6.1-8	8.1-10
<u>Amaranthus caudatus</u>	0	0	0	0
<u>Amaranthus paniculatus</u>	-	-	0	0
<u>Amaranthus tricolor</u>	-	-	-	-
<u>Celosia argentea f. plumosa</u>	-	-	-	0
<u>Leucaena leucocephala</u>	+	+	+	+
<u>Ocimum americanum</u>	-	-	0	0
<u>Phaseolus aureus</u>	0	0	0	0
<u>Phaseolus pubescens</u>	-	-	-	-
<u>Sesamum orientale</u>	-	-	-	-
<u>Sesbania bispinosa</u>	-	-	-	-
<u>Talinum paniculatum</u>	-	-	-	0
Non cultivated species				
<u>Ageratum conyzoides</u>	+	+	+	+
<u>Albizia lophantha</u>	0	+	0	0
<u>Amaranthus spinosus</u>	0	+	+	+
<u>Borreria brachystema</u>	+	+	+	+
<u>Cassia tora</u>	-	-	-	-
<u>Cleome gynandra</u>	-	-	-	-
<u>Crotalaria mucronata</u>	-	0	+	-
<u>Elaeagnus conforta</u>	0	0	+	+
<u>Hypericum leschenaultii</u>	-	-	-	-
<u>Hyptis brevipes</u>	+	0	0	-
<u>Impatiens javensis</u>	-	-	+	0
<u>Mycetia cauliflora</u>	-	-	-	-
<u>Myrsine affinis</u>	+	+	+	+
<u>Taraxacum officinale</u>	+	+	+	+

* Seeds are chilled in moist conditions, then brought to room temperature of 26° for 10 days illuminated by 500 lux fluorescent lamps for 15 hours per day. Percentage germination is same (0), higher (+), lower (-) of 5% (more) than control.

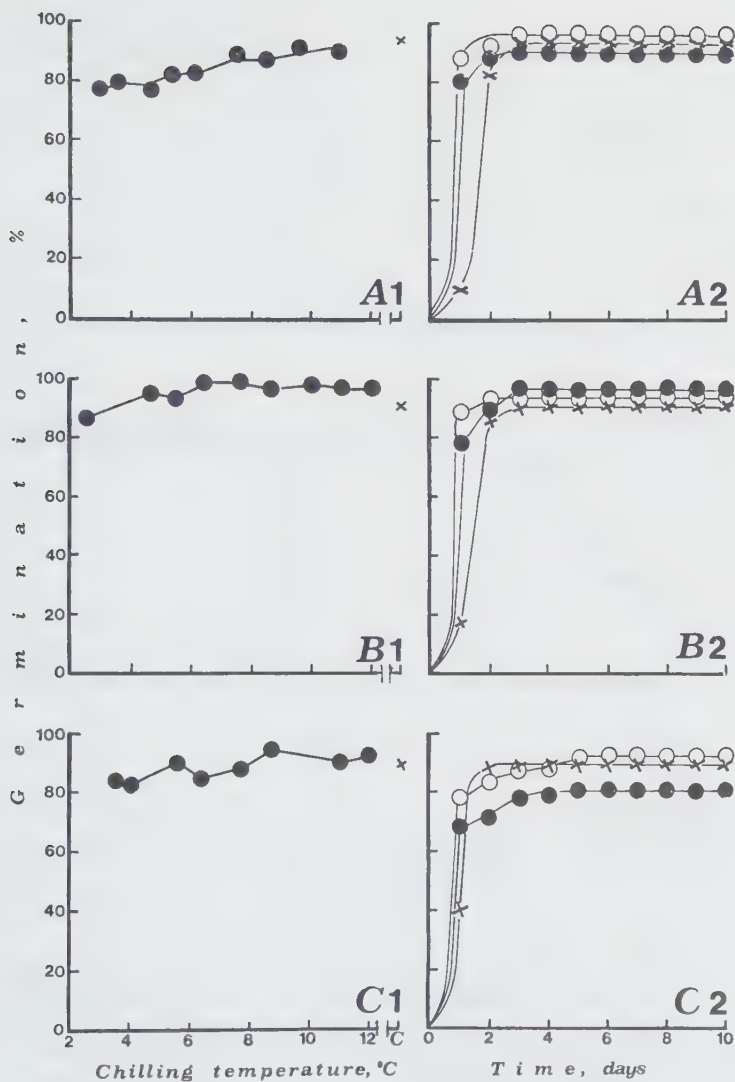


Fig. 13: Chilling response on germination in: A. *Amaranthus tricolor*, B. *Amaranthus paniculatus*, C. *Phaseolus aureus*.

- 1. chilling at various temperatures for 15 days, percentage germination is plotted against chilling temperature; treatment (●) and control (x).
- 2. chilling at 10°C for 10 days, percentage germination is plotted against time (days): chilling with pre-imbibition at 26°C for 5 hours (o), without pre-imbibition (●) and control (x).

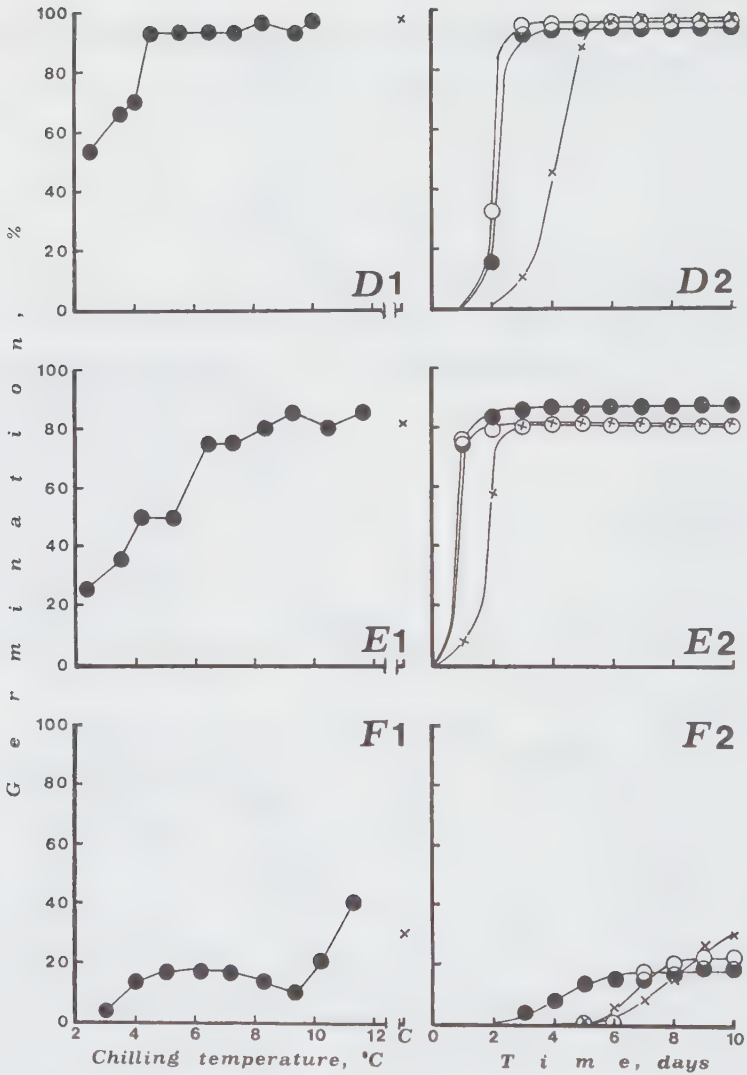


Fig. 13: (continued)
Chilling response on germination in Ocimum americanum (D), Celosia argentea (E), and Hypericum leschenaultii.
Legend is the same as previously.

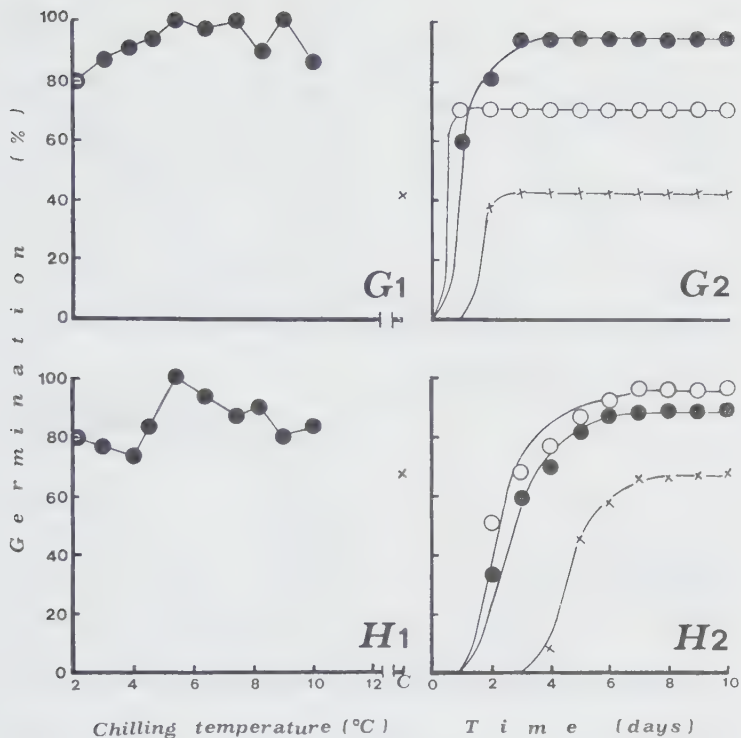


Fig. 13: (continued)

Chilling response on germination in *Borreria brachystema* (G) and *Ageratum conyzoides* (H).

Legend is the same as previously.

Communities of cultivated plants in tropical regions contain always in a varying proportion non cultivated plants. Their seeds are mostly dormant, therefore the effect of chilling in higher altitudes is more pronounced with the non cultivated plants which may be important weeds.

Fig. 13 shows that chilling pretreatment at any temperature between 2-10°C for 15 days under light of 50 lux fluorescent lamps promoted germination well in *Taraxacum officinale* (K1), *Borreria brachystema* (G1), *Amaranthus spinosus* (L1), *Ageratum conyzoides*

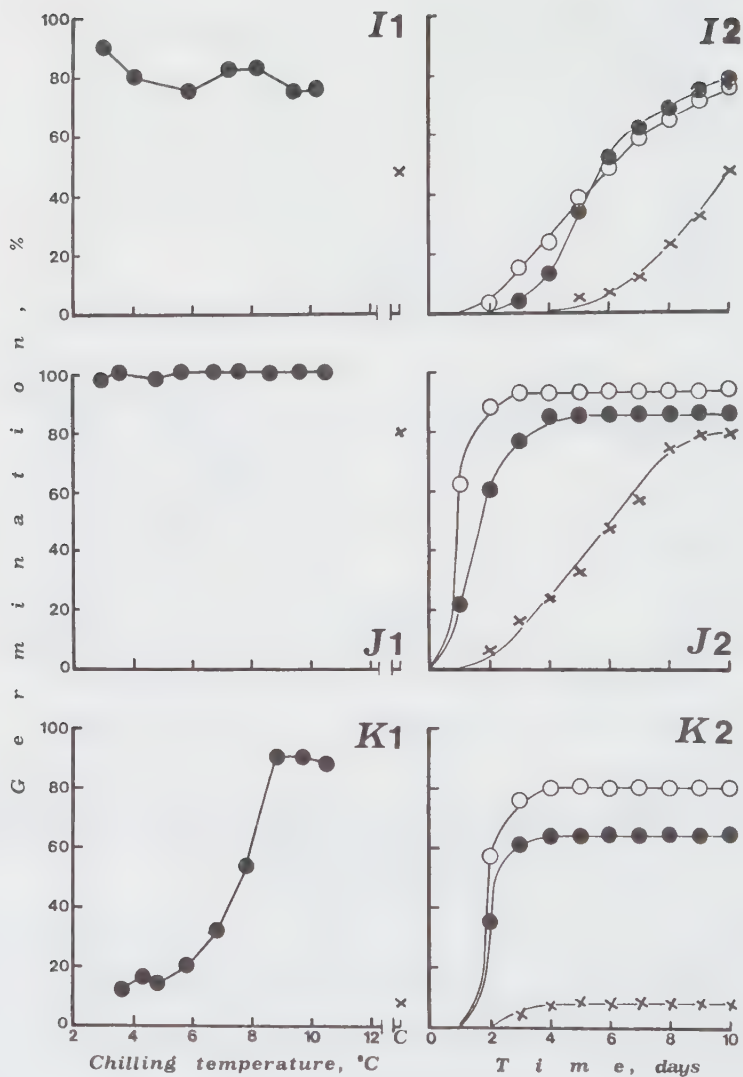


Fig. 13: (continued)

Chilling response on germination in *Leucaena leucocephala* (I), *Taraxacum officinale* (J) and *Amaranthus spinosus* (K). Legend is the same as previously.

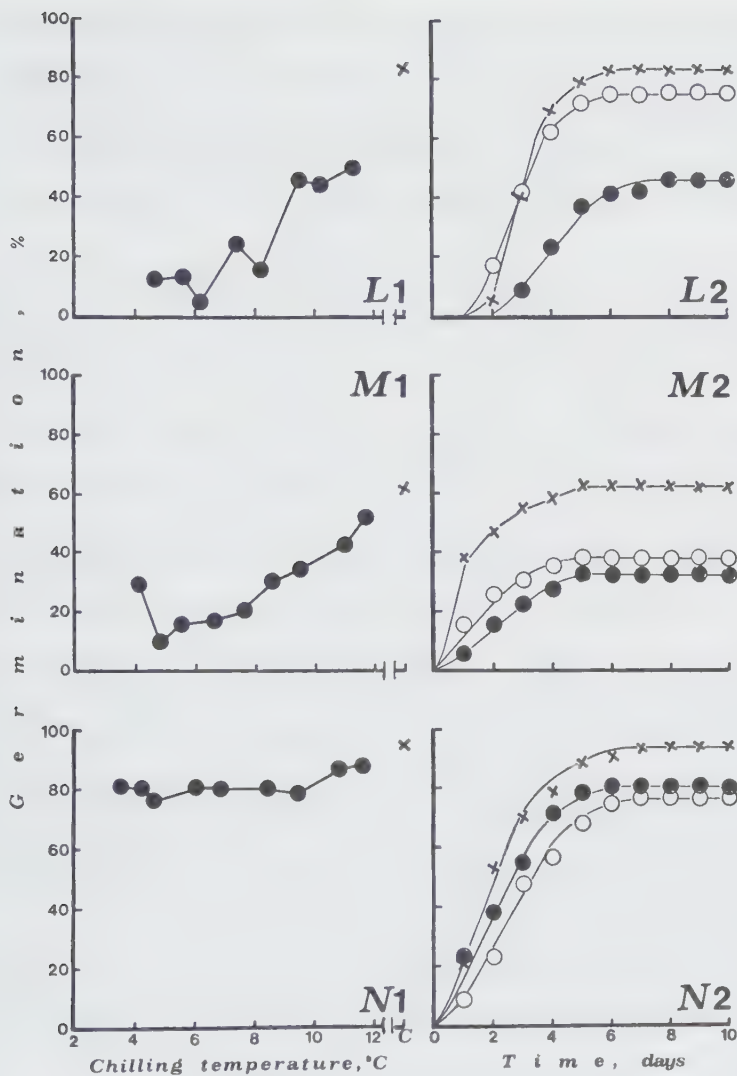


Fig. 13: (continued)
Chilling response on germination in *Sesamum orientale* (L), *Sesbania bispinosa* (M) and *Phaseolus pubescens* (N).
Legend is the same as previously.

(H1) and Leucaena leucocephala (J1). Chilling pretreatment at any temperature between $2.1-10^{\circ}\text{C}$ inhibits germination in Hypericum leschenaultii (F1), Phaseolus pubescens (D1), Sesbania bispinosa (N1) and Sesamum orientale (M1). The other species are inhibited or promoted by chilling only at certain temperatures.

Plants which can live at low temperatures and have a low optimal temperature for growth are termed psychrophiles. In fig. 14 the pictorial scatter diagram shows the interrelationship of five characters of germination: time and temperature for optimal germination, chilling response on germination (chilling with and without pre-imbibition) and cultivation status of species. The optimum temperature of germination associates obviously with speed of germination. The higher the optimum temperature of germination, the quicker the seed germinate. A positive response of germination to chilling treatment is stronger with species having low optimum temperatures than with species having high optimum temperatures. Cultivated species in general have high optimum temperatures and they are not promoted by chilling treatments with or without pre-imbibition. In contrast non cultivated species have low optimum temperatures and they have higher chances to be promoted by chilling treatments either with or without pre-imbibition.

Amaranthus spinosus (no. 4) differs obviously from the other species, because its germination characteristics are extreme in the groups of non cultivated or cultivated plants.

In respect of its optimum temperature of germination, this species is however similar to most cultivated species. This may be one of the reasons for the common occurrence of A. spinosus in tropical crop fields. It is interesting to note that by exposure to low temperature of about 10°C before germination the number of seedlings

of Amaranthus spinosus increases abundantly, compared with germination in normal conditions. When this situation occurs in crop fields of Celosia argentea f. plumosa (no. 7), Amaranthus caudatus (no. 2), Amaranthus paniculatus (no. 3), Sesbania bispinosa (no. 16), Phaseolus aureus (no. 13) and Phaseolus pubescens (no. 14), the seedling proportion of Amaranthus spinosus in these populations becomes more competitive, because germination of these crop plants is not affected by a chilling treatment with or without pre-imbibition.

In areas with a temperate climate most seeds need a minimal period of chilling for germination. The natural mechanism for breaking dormancy in these seeds is simply moist winter chilling. Exposure to low temperature in moist condition before germination is known as stratification.

The pre-imbibition treatment at 26°C for 5 hours under continuous light before chilling (Fig. 13) promotes germination significantly (at 99% level) in Ageratum conyzoides (H2), Borreria brachystema (G2) and Amaranthus spinosus (L2), it inhibits however germination significantly (at 99% level) in Phaseolus pubescens (O2) and Cleome gynandra (Fig. 14, no. 8). This pre-imbibition treatment is useful for overcoming an inhibition by a chilling treatment in Sesamum orientale (M2) and Sesbania bispinosa (N2). These observations may be significant for either agricultural or physiological investigations.

Stratification of Amaranthus spinosus at 14°C for 7 days increases the speed and uniformity of the germination response to temperature (Fig. 15).

As regards the mechanism of stratification the work of Baron (1970) about the energy relationships of the embryo and gametophyte of

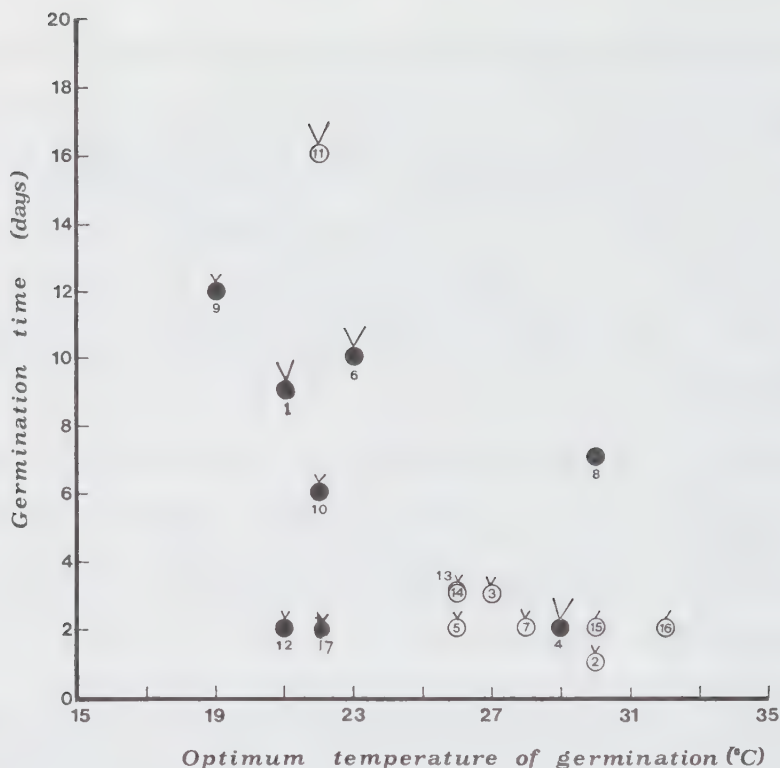


Fig. 14: Pictorial scatter diagram showing interrelationships of five characters: optimum temperature and time of germination, cultivation, sensitivity to chilling with and without pre-imbibition among some tropical species. Cultivation: O: cultivated, ●: non cultivated Chilling sensitivity:

without		with
○ : promoted at 99% level :		○
○ : indifferent :		○
○ : inhibited at 99% level :		○

- | | |
|-----------------------------------|----------------------------------|
| 1. <u>Ageratum conyzoides</u> | 10. <u>Hyptis brevipes</u> |
| 2. <u>Amaranthus caudatus</u> | 11. <u>Leucaena leucocephala</u> |
| 3. <u>Amaranthus paniculatus</u> | 12. <u>Ocimum americanum</u> |
| 4. <u>Amaranthus spinosus</u> | 13. <u>Phaseolus aureus</u> |
| 5. <u>Amaranthus tricolor</u> | 14. <u>Phaseolus pubescens</u> |
| 6. <u>Borreria brachystema</u> | 15. <u>Sesamum orientale</u> |
| 7. <u>Celosia argentea</u> | 16. <u>Sesbania bispinosa</u> |
| 8. <u>Cleome gynandra</u> | 17. <u>Taraxacum officinale</u> |
| 9. <u>Hypericum leschenaultii</u> | |

sugar pine (Pinus lambertiana) at high and low temperatures is relevant. At low temperatures the mobilization of nutrients exceeds their utilization, leading to a temporary accumulation. This condition finds its expression in an increased growing power as reported by Asakawa (1959) for Pinus and by Pollock and Olney (1959) for Prunus cerasus. Willemsen (1975) noted in Ambrosia artemisiifolia seeds that as the duration of stratification was increased at any temperature, more seeds became able to germinate at a wider range of temperatures. Other workers (Villiers, 1975; Stone, 1957) explained the effect of low temperatures in breaking dormancy with a changed restriction of the oxygen diffusion through the seed coats. At warm temperatures oxygen consumption will be high and the seeds may suffer from a lack of oxygen causing certain metabolic pathways to be changed. However, if the seeds are maintained at temperatures of about 5°C the rate of respiration will be greatly slowed down and the amount of oxygen diffusing inwards might now be sufficient to allow a diversion of these pathways back to those allowing growth.

Many workers (Davis & Rose, 1912; Eckerson, 1913; Roberts, 1924; Flemion, 1931; Haut, 1933; Choate, 1940) have shown freezing temperatures of -3 and -5°C to be ineffective in breaking dormancy in Crataegus mollis, Sorbus aucuparia, Echinocystis lobata and a species of Ferula. A relatively weak chilling to 10°C during imbibition damages seeds of Pinus lunatus (Dogras et. al., 1977). He showed that chilling sensitive lima beans did not exhibit any change in fatty acid composition from imbibition to the 6th day of germination when kept at 10°C. In contrast in the chilling-resistant plants, there was a shift in the metabolism toward the production of fatty acids. It may be concluded that the seeds are probably injured by low temperature preventing the shift in fatty acid metabolism. This

agrees with the concept that exposure to low temperatures generally causes shifts in lipid metabolism toward more unsaturated fatty acids (De La Roche et al, 1973; Hoelzl & Wagner, 1971; Shewry et al, 1973).

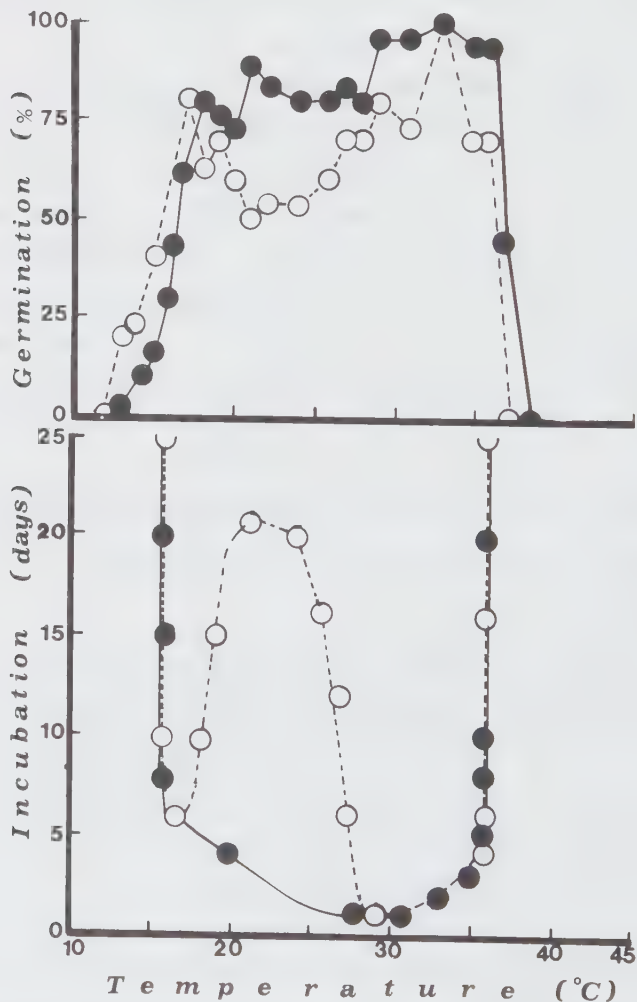


Fig. 15: Germination curves of Amaranthus spinosus at a thermogradient bar. Above: percentage germination is recorded in period of incubation for 25 days, plotted against germination temperature. Below: successive days for percentage germination of 50% is plotted against temperature of germination. Comparison of germination behavior of seed populations before (O) and after stratification at 14°C for 7 days (●).

3.7. Response of heating on germination

The most common means of reducing the "hardness" of seed has been mechanical scarification. Seed dormancy by germination inhibitors and hard seed coats is also broken more rapidly at high temperatures in moist condition (Mitchell, 1970). It has been reported that hot water treatment not only enhanced inhibition but also proved helpful in increasing the germinability of seeds of Prosopis juliflora (Chatterjii & Mohnot, 1968). According to Shetty (1968), a high temperature (30° - 40° C) is required by the seeds of Biophytum sensitivum in later months of the rest period to break dormancy.

In a series of experiments with exposure to 40° C in moist condition before germination in normal temperature (26° C) varied responses were obtained (Fig. 16). Percentage germination of Leucaena leucocephala and Phaseolus aureus is significantly promoted (at 99% level) by a heating treatment for one to five days. In Amaranthus spinosus and Cleome gynandra, germination was significantly promoted by heating treatment for one to four days. Borreria brachystema needs a heating treatment for 2 to 5 days to promote significantly germination. Sesbania bispinosa and Hyptis brevipes are promoted significantly by a heating treatment for 4 and 5 days respectively.

Germination of some species is significantly inhibited (at 99% level) by a heating treatment: Amaranthus paniculatus by a heating treatment for 1-5 days, Hypericum leschenaultii and Impatiens javensis by a treatment for more than one day. The germination of Mycetia cauliflora is inhibited only when the heating treatment lasts 4 days or longer. The seeds of Cassia tora show an inhibition of germination when treated for 5 days.

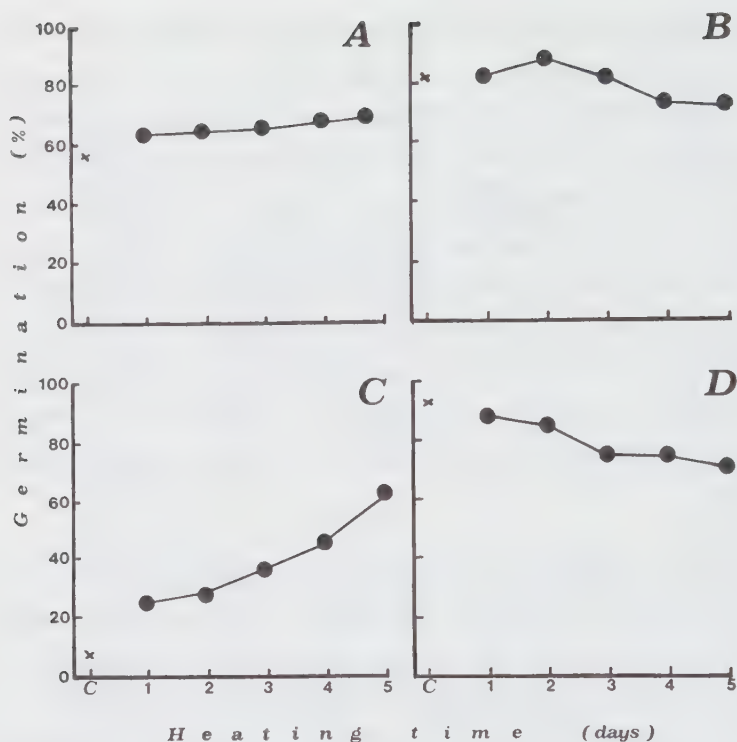


Fig. 16: Heating response on germination of Phaseolus pubescens (A), Sesamum orientale (B), Leucaena leucocephala (C) and Amaranthus paniculatus (D) Percentage germination is plotted against time period of heating treatment. C(x): control.

An indifferent response of germination to a heating treatment for even 5 days is shown by Ageratum conyzoides, Crotalaria mucronata, Phaseolus pubescens, Sesamum orientale, Celosia argentea f. plumosa, Amaranthus tricolor, Talinum paniculatum and Ocimum americanum (Table 12 and 13).

A requirement for high temperatures to remove dormancy in the seeds may be one of the causative factors for the species to occur only in the tropics.

Table 12: Heating response of germination: cultivated species

Species	Period of heating (days)*				
	1	2	3	4	5
<u>Amaranthus paniculatus</u>	-	-	-	-	-
<u>Amaranthus tricolor</u>	0	0	0	0	0
<u>Celosia argentea f. plumosa</u>	0	0	0	0	0
<u>Leucaena leucocephala</u>	+	+	+	+	+
<u>Ocimum americanum</u>	0	0	0	0	0
<u>Phaseolus aureus</u>	+	+	+	+	+
<u>Phaseolus pubescens</u>	0	0	0	0	0
<u>Sesamum orientale</u>	0	0	0	0	0
<u>Sesbania bispinosa</u>	0	0	0	+	0
<u>Talinum paniculatum</u>	0	0	0	0	0

* Heating at 40°C of moistened seeds for 0 (control) 1, 2, 3, 4 and 5 days, then seeds transferred to germinate at 26°C for 10 days; indifferent (0), inhibited (-) and promoted (+) at 99% level against control.

Table 13: Heating response of germination: non cultivated species

Species	Mean ** altitude of distri- bution (m)	Period of heating* (days)				
		1	2	3	4	5
<u>Hypericum leschenaultii</u>	2391	0	-	-	-	-
<u>Impatiens javensis</u>	1559	0	-	-	-	-
<u>Mycetia cauliflora</u>	1420	0	0	0	-	-
<u>Crotalaria mucronata</u>	500	0	0	0	0	0
<u>Ageratum conyzoides</u>	459	0	0	0	0	0
<u>Borreria brachystema</u>	325	0	+	+	+	+
<u>Hyptis brevipes</u>	318	0	0	0	0	+
<u>Amaranthus spinosus</u>	287	+	+	+	+	0
<u>Cleome gynandra</u>	225	+	+	+	+	0
<u>Cassia tora</u>	167	0	0	0	0	-

* Same experimental conditions as for table 12.

** Based on herbarium material study and literature.

Table 13 shows a relation between heating resistance and mean altitude of distribution of non cultivated species from Java. It can clearly be seen that the higher the mean altitude, the weaker the heating resistance of the species. The relation suggests that exposure to high temperatures in moist conditions promotes germinability of the seed of most species from lowlands, but inhibits most species growing at higher altitudes.

3.8. Effects of storage conditions on germinability

Germination ability at constant temperature is affected by the duration of storage. Dry seed (moisture content 7%) of Amaranthus paniculatus which were stored in sealed containers at 65⁰C for 106 days (Fig. 17), showed a narrower temperature span of germination than before storage. Germination ability at high and low temperatures is lost by storage. A further effect is the reduction of germination speed at any temperature. The optimum temperature of germination (25⁰C) is slightly different to that of seeds before storage (25-29⁰C), but the time for germination at the optimum temperature (12 days) is obviously longer than with seeds before storage (3 days). Fig. 18 shows the germination behaviour of Capsicum annuum (seed moisture content 9%) after storage at 40⁰C for 177 days with prestorage 50⁰C for 3, 4 and 5 days. The temperature spans of germination in stored seeds with a prestorage for 3 and 4 days are not different, but with a prestorage for 5 days the span is narrower (about 12 to 30⁰C) than in seeds with a prestorage of 3 and 4 days. Among these three variants of stored seeds, the optimum temperatures of germination are not different (25⁰C), but germination at the optimum temperature begins one

day later in seeds with prestorage for 5 days than in seeds with a prestorage for 3 and 4 days.

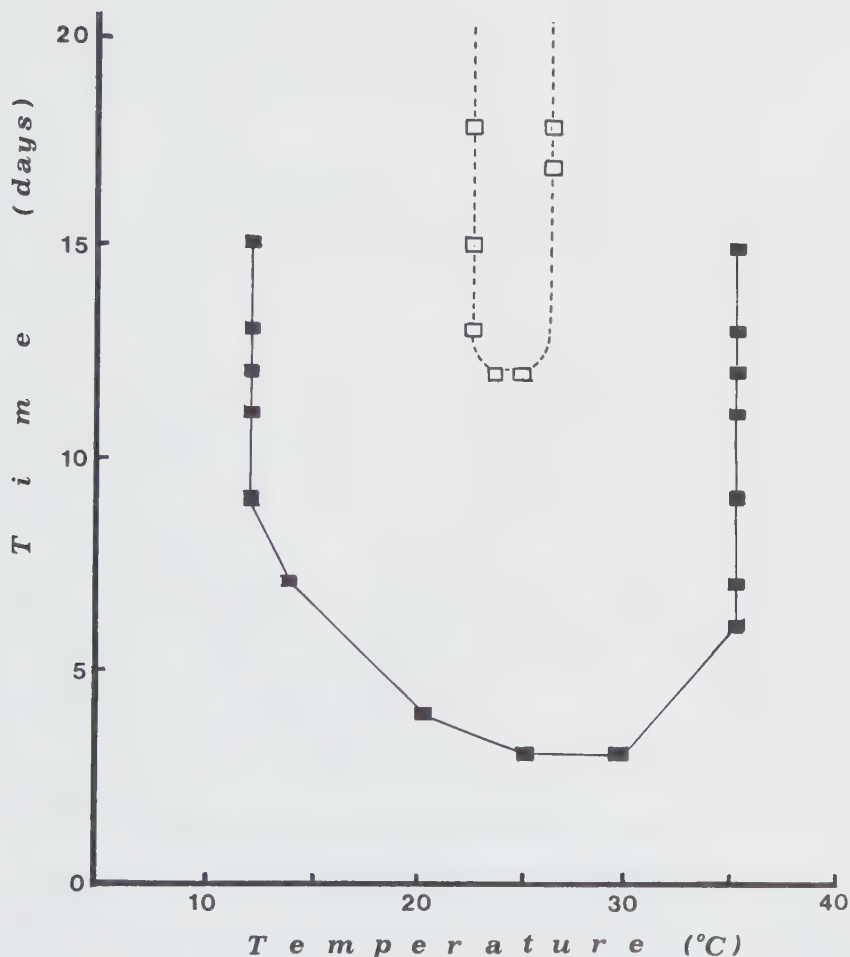


Fig. 17: Effect of duration of storage on cardinal temperatures of germination of *Amaranthus paniculatus* seeds. Storage in sealed containers at 65°C for 106 days □. Germination curve before storage ■. (Seed moisture content 7%)

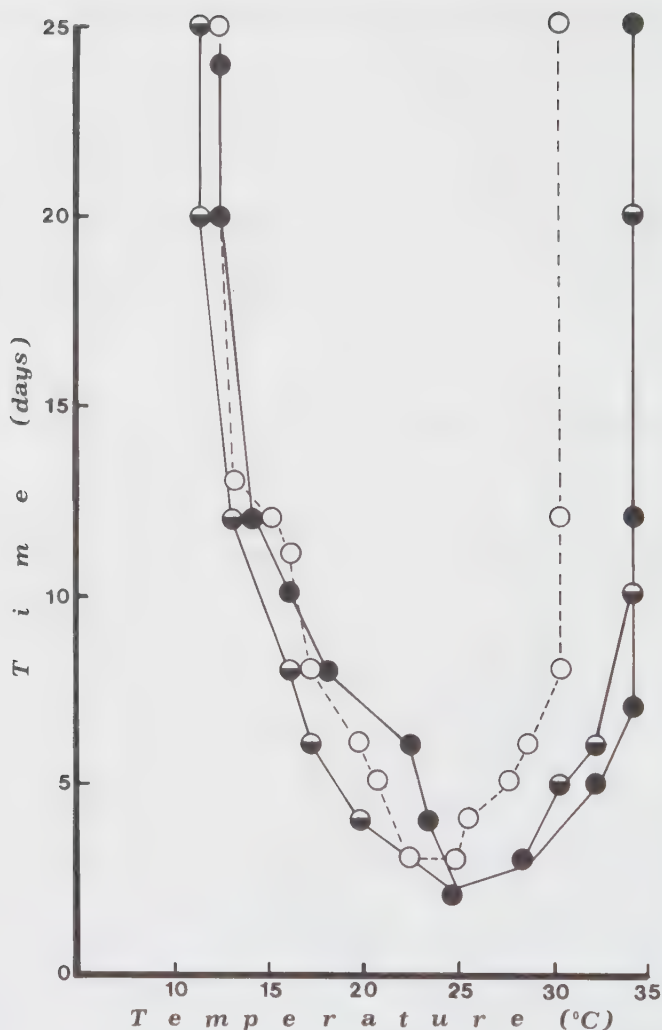


Fig. 18: Effect of storage in sealed containers at 40°C for 177 days of *Capsicum annum* seeds (moisture content 9%) on cardinal temperatures of germination. Treatment of prestorage at 50°C for 3 (●), 4 (●) and 5 (○) days. Germination on the thermogradient bar at 0-50°C. The successive days for germination of 50% were plotted against temperature. The temperature corresponding to the least number of days for 50% germination is considered to be the optimum temperature of germination.

Fig. 19 shows the chilling sensitivity of Amaranthus paniculatus seeds (moisture content of 7%) from stocks before and after storage in sealed containers at 65°C for 106 days. Exposure to a chilling treatment at 2-10°C before germination in normal condition does not influence percentage germination in seeds from stock before storage, but inhibits obviously germination of seeds from stock after storage at a high temperature.

Exposure to a heating treatment at 40°C for 1-5 days before germination in normal condition inhibits percentage germination of seeds from stock after storage with germinability lower than from stock before storage.

The effect of a high temperature prestorage period on chilling sensitivity of Capsicum annuum seeds (moisture content 9%) has been studied. Three stocks of seeds were prepared by storing seeds in sealed containers at 40°C for 177 days with a prestorage at 50°C for 3, 4 and 5 days. Fig. 20 shows the results of chilling on germination with the different prestorage period. Chilling at 8-10°C in moist conditions before germination in normal condition inhibits obviously germination when the seeds were heated for 5 days to 50°C, but only slightly with 3 and 4 days prestorage. Chilling treatment at 2-8°C for 15 days inhibits strongly germination of all three stocks.

The storage experiments with Amaranthus paniculatus seeds show clearly that germinability and germination vigour may be reduced by high temperature storage. The experiment suggests that regeneration of seeds must be carried out regularly in long-term storage of seeds. It has been reported that the effect of storage on loss of viability of seed is caused by chromosome aberrations (Navashin, 1933a, b; Peto, 1933), possibly leading also to increas-

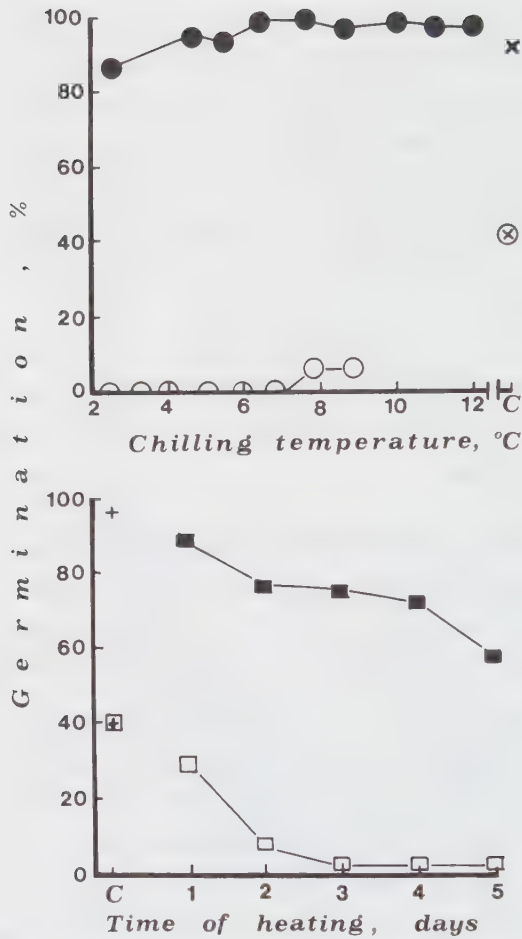


Fig. 19: Effect of duration of storage in sealed containers at 65°C for 106 days of Amaranthus paniculatus seeds on chilling and heating sensitivity.

Prechilling at various temperatures, then seeds germinated at 26°C for 10 days.

- : germination with prechilling of the seeds without storage treatment
 - × : germination without prechilling of the seeds without storage treatment
 - : germination with prechilling of the seeds with storage treatment
 - ⊕ : germination without prechilling of the seeds with storage treatment
- Heating sensitivity: preheating was done at 40°C for 0-5 days, then seeds brought to germinate at 26°C for 10 days.
- : germination with preheating of the seeds without storage treatment
 - ⊕ : germination without preheating of the seeds without storage treatment
 - : germination with preheating of the seeds with storage treatment
 - ⊕ : germination without preheating of the seeds with storage treatment
- (C: control)

ing rates of pollen abortion mutations (Cartledge & Blakeslee, 1933, 1934). Further investigations by Blakeslee & Avery (1934) showed that a high rate of phenotypic mutations was also induced by aging in seeds. Work of Roberts, Abdalla and Owen (1967) proved also the relationship between percentage viability and the mean frequency of aberrant cells in the surviving seeds of broad bean. One further minor implication is that deliberately 'poor' storage conditions could be used as a simple method for inducing mutations in breeding work. In other cases certain condition of a prestorage treatment which does not harm germinability, viability and germination vigour may be used as a method for reducing seed coat pathogens before the seeds are stored.

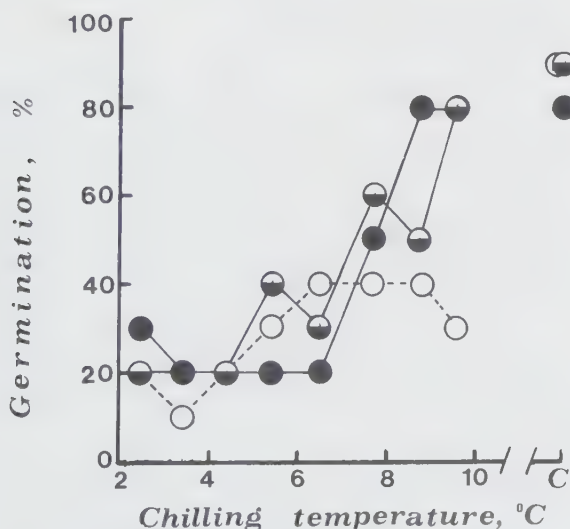


Fig. 20: Effect of high temperature prestorage on chilling sensitivity of *Capsicum annuum* seeds. Seeds were stored in sealed containers at 40°C for 177 days with prestorage at 50°C for 3 (●), 4 (●) and 5 (○) days. Firstly seeds were chilled at various temperatures between 2 and 10°C for 15 days under light of 50 lux fluorescent lamps, then brought to germinate at 26°C for 10 days under light of 500 lux fluorescent lamps for 15 hours per day. C = control (without chilling treatment).

3.9. Control of seed viability

It has long been known that the major factor which influence the longevity of seeds in storage are temperature, moisture content and oxygen pressure (Owen, 1956; Barton, 1961; James, 1967). According to Roberts and Ellis (1977) at present best estimates of regeneration interval of seeds in storage are obtained by applying the three viability equations and the four viability constants determined at higher temperature to predict the time taken for viability to fall by 5% at -6°C (see 2.9.3.). By following that suggestion, the viability constants of nine cultivated species have been determined. The value of K_{σ} varies from 0.061 to 0.245; K_v varies from 4.119 to 6.119; c_1 and c_2 vary from 0.041 to 0.134 and 0.020 to 0.061 respectively (Table 14).

Vigna unguiculata var. cylindrica (Fig. 21), Sesbania bispinosa (Fig. 22), Phaseolus pubescens (Fig. 23), Leucaena leucocephala (Fig. 24) and Phaseolus aureus (Fig. 25) have a low value of K_{σ} (lower than 0.20). This constant determines the slope of the survival curve which is a measure of the seed to seed variation in longevity. Therefore when the seeds of these species are stored in constant conditions of temperature and moisture content, a drastic loss of viability is predicted at the mean viability time. This suggests that at around the time of the mean viability period of the seeds, monitoring viability tests must more often be carried out. On the other hand a high value of K_{σ} in Vigna unguiculata var. sinensis (Fig. 26), Cajanus cajan (Fig. 27), Amaranthus tricolor (Fig. 28) and Sesamum orientale (Fig. 29) indicates that the loss of viability at around the time of the mean viability period falls gradually. Therefore monitoring the viability loss in these species can be made at greater intervals.

Table 14: Probable regeneration intervals for seed stored at -6°C and 5% moisture content

Species	Seed color	Viability constants			Interval* (years)
		K_{σ}	K_V	c_1 c_2	
<u>Amaranthus tricolor</u>	black	0.215	6.119	0.131	0.044
<u>Sesbania bispinosa</u>	greenish yellow	0.099	5.603	0.088	0.059
<u>Phaseolus pubescens</u>	violet	0.085	5.674	0.118	0.059
<u>Vigna unguiculata var. cylindrica</u>	brown	0.135	5.464	0.120	0.048
<u>Vigna unguiculata var. sinensis</u>	brown	0.245	5.546	0.134	0.055
<u>Cajanus cajan</u>	black	0.197	5.405	0.124	0.061
<u>Leucaena leucocephala</u>	brown	0.061	4.119	0.041	0.046
<u>Sesamum orientale</u>	white	0.211	4.935	0.198	0.041
<u>Phaseolus aureus</u>	green	0.090	4.556	0.195	0.020

* Probable regeneration interval is calculated as predicted time for viability to fall to 95% of initial value.

The influence of prestorage treatments on the value of K_G has been investigated in seeds of Leucaena leucocephala, Phaseolus aureus and Sesamum orientale (Table 15). Prestorage at 50°C for 0 (control), 3, 4 and 5 days and then storage at 40°C results in equal values of K_G of Phaseolus aureus seeds with moisture content of 8%, slightly different values for Sesamum orientale seeds with moisture content of 5%. Under conditions of prestorage at 60°C for 0 (control), 3, 4 and 5 days, subsequent storage at 50°C results in no difference in K_G for Leucaena leucocephala seeds with moisture content of 6%. These results suggest that K_G is quite suitable as the genotypic constant of viability. Roberts and Ellis (1977) reported that for cultivars of barley (Hordeum distichum) the values of c_1 and c_2 are identical. Genotypic differences in longevity are attributable to differences in the value of the K constants: K_G varies only slightly but there are quite large differences in K_V . In further investigations on factors which could affect subsequent viability, including nitrogen nutrition, shading, degree of

Table 15: Influence of prestorage treatment on the value of K_G

	Time of prestorage (days)			
	0	3	4	5
<u>Phaseolus aureus</u>				
moisture content 8%				
prestorage 50°C				
storage 40°C	0.073	0.083	0.066	0.069
<u>Sesamum orientale</u>				
moisture content 5%				
prestorage 50°C				
storage 40°C	0.307	0.116	0.103	0.154
<u>Leucaena leucocephala</u>				
moisture content 6%				
prestorage 60°C				
storage 50°C	0.040	0.056	0.059	0.065

unripeness and severity of threshing, it was reported that only shading and marked unripeness significantly affected longevity. The value of K_v was reduced considerably in contrast to K_o .

Table 14 shows the predictions of the longevity for seed viability to fall to 95% of the initial value by storing at -6°C with seed moisture content of 5%. For the different species, a relationship between the value of K_v and the period of viability is obvious. The higher the value of K_v , the longer is the period of viability. According to Roberts and Ellis (1977) the value of K_v is affected by both genotypic and prestorage environmental factors. Accurately determined viability constants (K_o , K_v , c_1 and c_2) for the basic viability equations are important, because they permit to define percentage viability after any period of storage in any combination of conditions.

As the validity of the three viability equations has been proved for a few species only these results must be regarded with some caution. Much further work is needed to establish firm rules for the relation between storage conditions and longevity of seed.

The relationship between oxygen pressure and the period of viability has been discussed in detail (Roberts, 1972). From more recent work on the influence of the gaseous environment, it has emerged that there is an inverse relationship between pressure of oxygen and period of viability.

The presence of microorganism which is closely related to temperature and humidity influences also seed longevity. At this time there are no satisfactory chemical methods for the control of microorganism in storage. The evidence indicates that all storage fungi are completely inactive below 65% relative humidity. Mites can not

survive below 60% relative humidity and they tend to die out between 60 and 70%; multiplication is rapid above 75% humidity (Oxley, 1948). Using bacteria for the biological control of insects in seed storage is possible, but their effect in seeds used as food must be investigated. Difficulties may also arise when large amounts of seeds are stored.

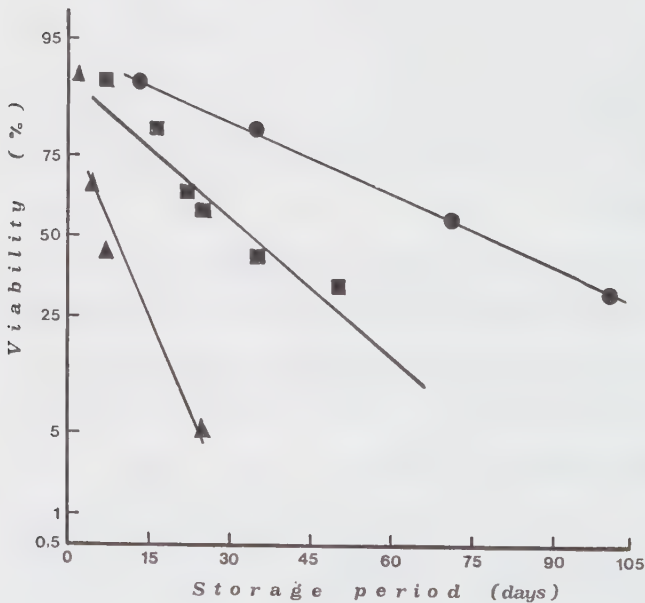


Fig. 21: Survival curves of *Vigna unguiculata* var. *cylindrica* seeds in hermetic storage under 3 combinations of temperature and moisture content (mc): 11% mc, 47°C (●); 14% mc, 47°C (■); 14% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 20 seeds each) is plotted on probability scale against storage period.

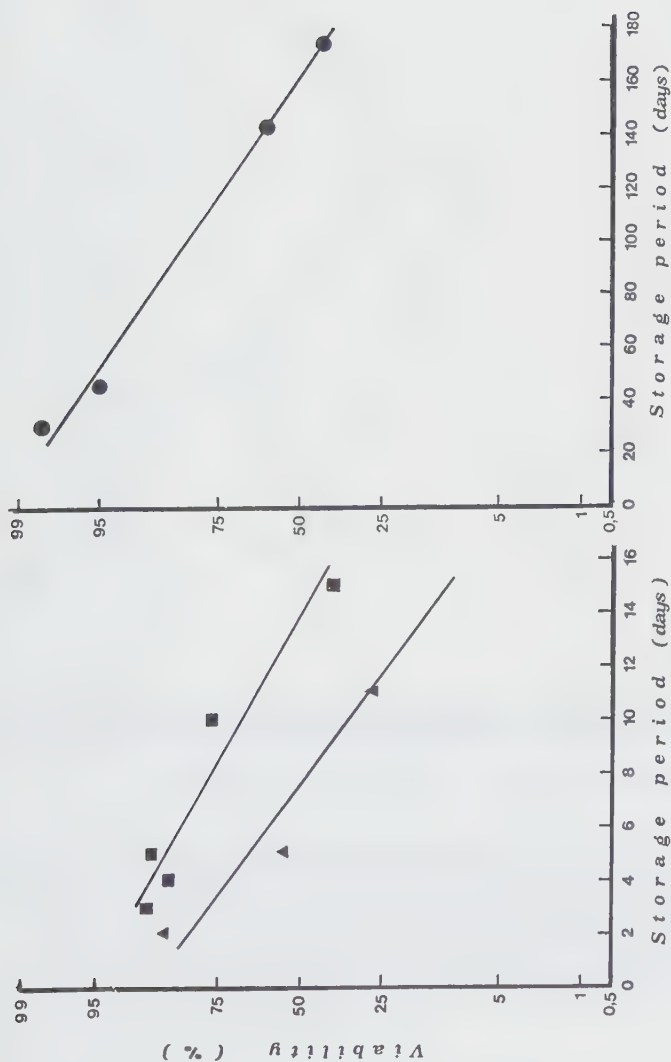


Fig. 22: Survival curves of *Sesbania bispinosa* seeds in hermetic storage under 3 combinations of temperature and moisture content: 12% mc, 40°C (●); 12% mc, 58°C (■); 14% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 30 seeds each) is plotted on probability scale against storage period.

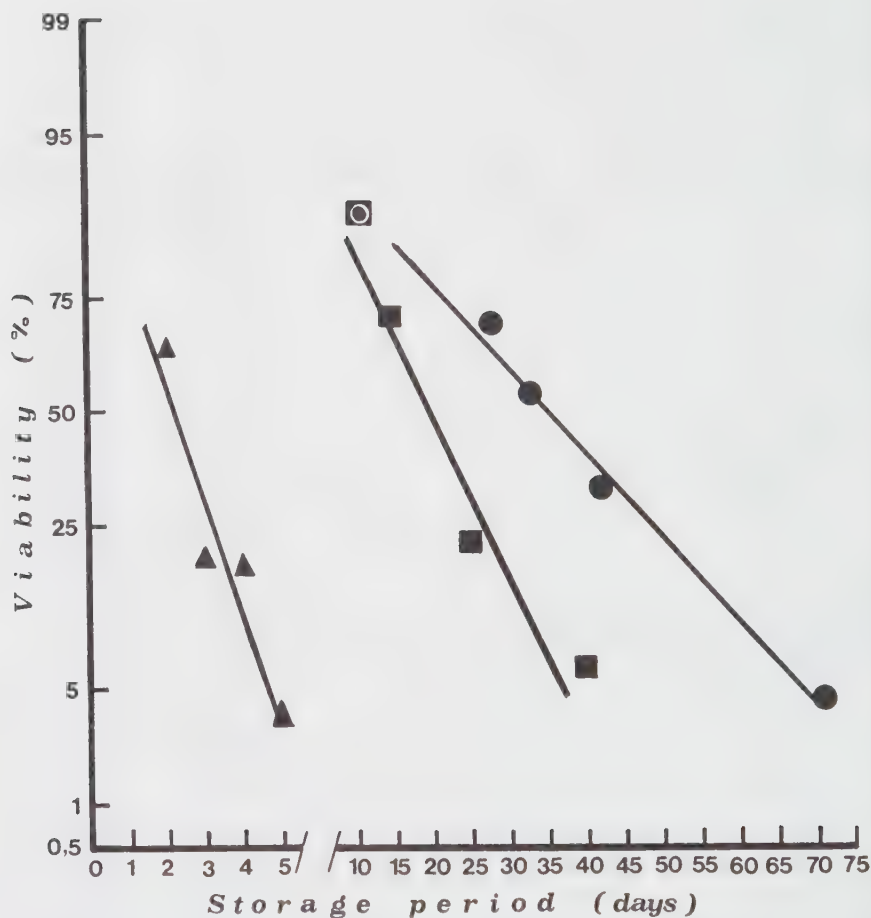


Fig. 23: Survival curves of *Phaseolus pubescens* seeds in hermetic storage under 3 combinations of temperature and moisture content: 15% mc, 40°C (●), 17% mc, 40°C (■), 15% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 30 seeds each) is plotted on probability scale against storage period.

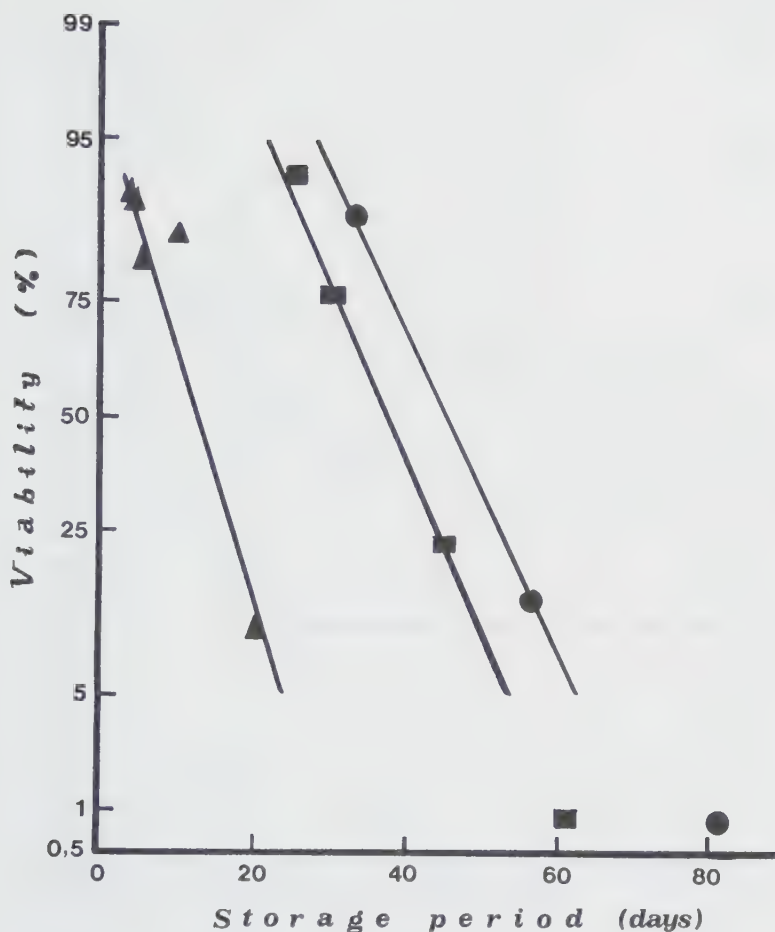


Fig. 24: Survival curves of *Leucaena leucocephala* seeds in hermetic storage under 3 combinations of temperature and moisture content: 4% mc, 50°C (●), 6% mc, 50°C (■), 6% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 30 seeds each) is plotted on probability scale against storage period.

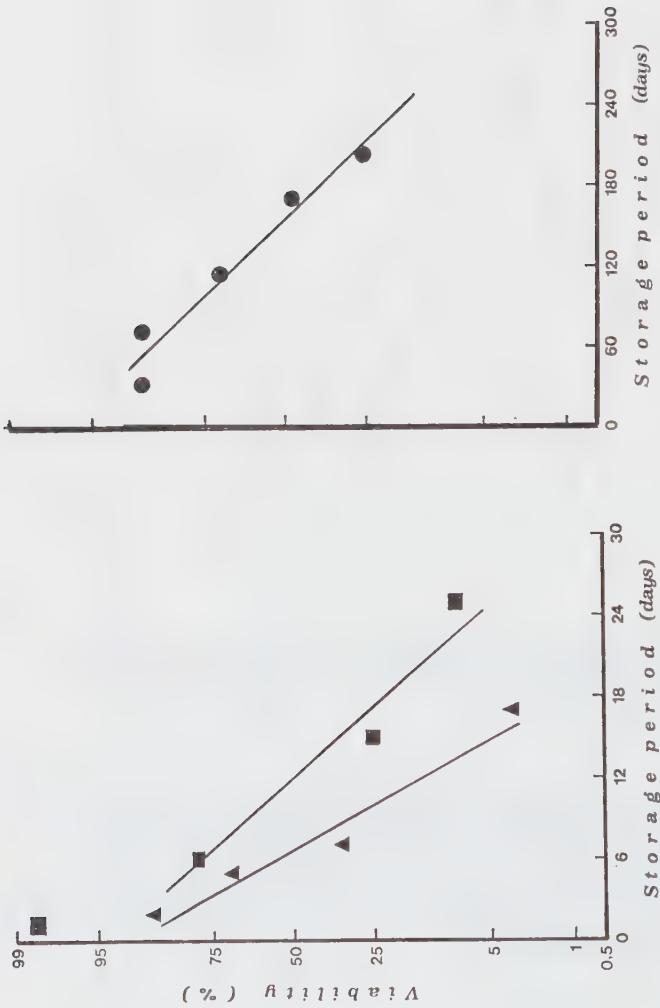


Fig. 25: Survival curves of *Phaseolus aureus* seeds in hermetic storage under 3 combinations of temperature and moisture content: 8% mc, 40°C (●); 13% mc, 47°C (■); 13% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 30 seeds each) is plotted on probability scale against storage period.

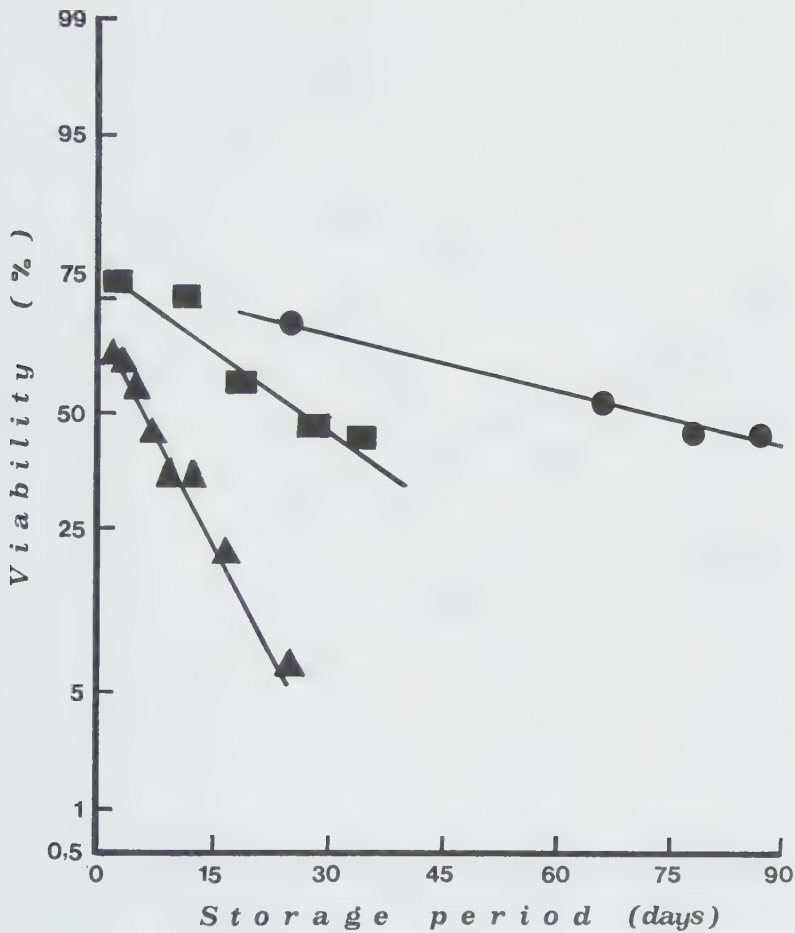


Fig. 26: Survival curves of *Vigna unguiculata* var. *sinensis* seeds in hermetic storage under 3 combinations of temperature and moisture content: 11% mc, 40°C (●), 6% mc, 60°C (■), 11% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 15 seeds each) is plotted on probability scale against storage period.

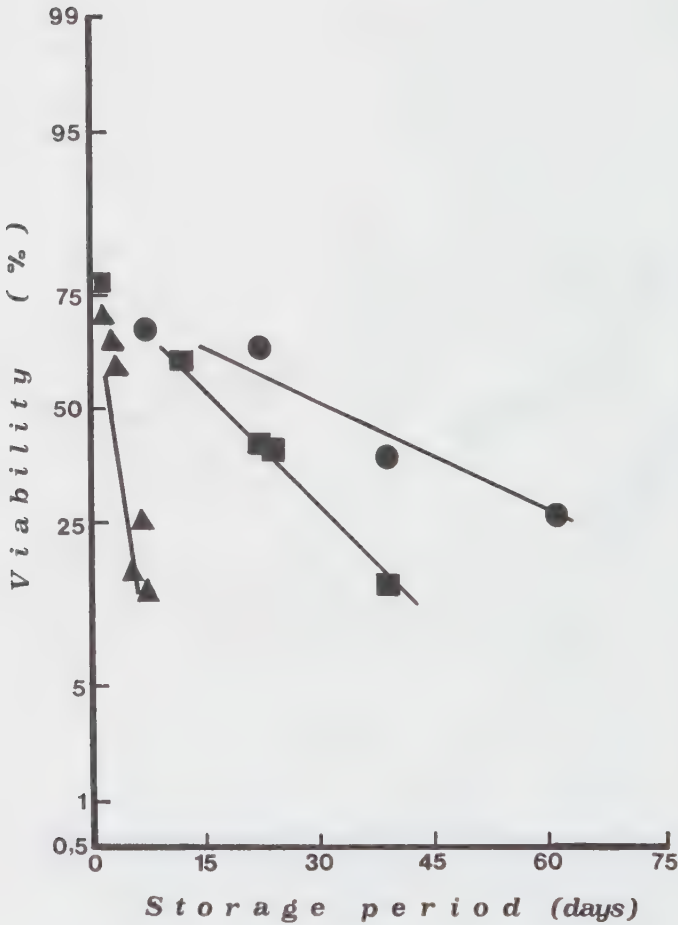


Fig. 27: Survival curves of *Cajanus cajan* seeds in hermetic storage under 3 combinations of temperature and moisture content: 12% mc, 40°C (●), 14% mc, 40°C (■), 12% mc, 60°C (▲). Percentage viability (mean of 4 replicates of 30 seeds each) is plotted on probability scale against storage period.

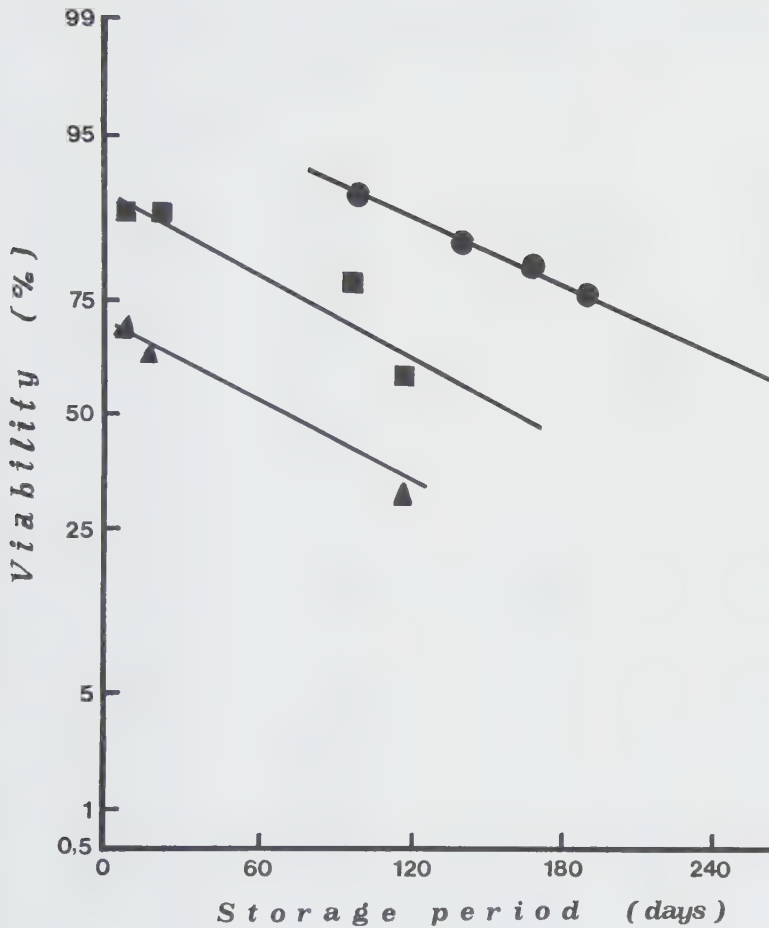


Fig. 28: Survival curves of *Amaranthus tricolor* seeds in hermetic storage under 3 combinations of temperature and moisture content: 11% mc, 50°C (●), 13% mc, 50°C (■), 13% mc, 60°C (▲). Percentage viability (mean of 5 replicates of 50 seeds each) is plotted on probability scale against storage period.

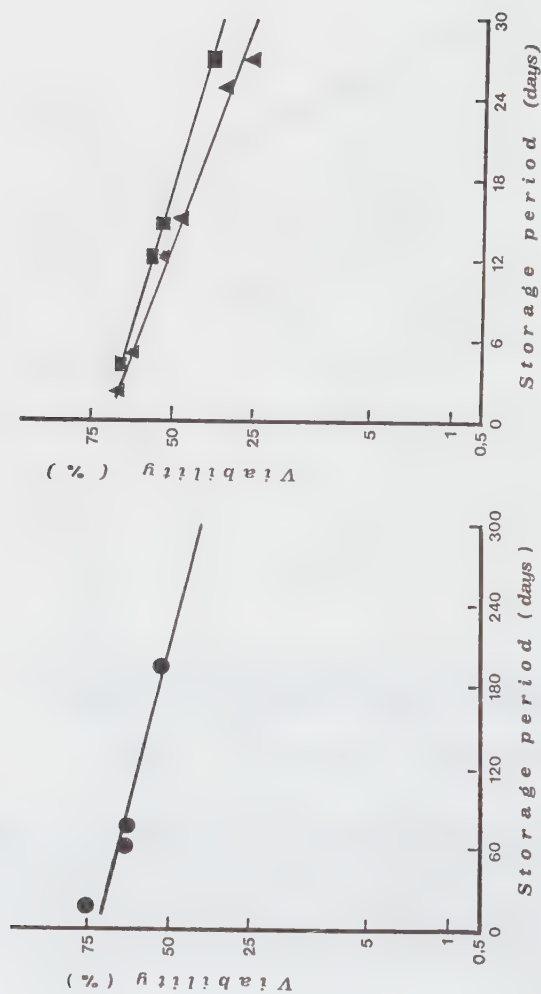


Fig. 29: Survival curves of *Sesamum orientale* seeds in hermetic storage under 3 combinations of temperature and moisture content: 5% mc, 40°C (O), 9% mc, 47°C (■), 7% mc, 60°C (▲). Percentage viability (mean of 5 replicates of 50 seeds each) is plotted on probability scale against storage period.

Table 15: List of species with their regional and altitudinal distribution in Java based on herbarium material (of Herbarium Bogoriense, Indonesia) and literature.

Ageratum conyzoides

Location	Altitude (m)	Herbarium number
Pelabuhan Ratu	1	34650
Pasuruhan	3	7682
Puger, Jember	5	20564
Malingping, Banten	5 - 40	1424
Sentiong, Jakarta	10	21339
Pegangsaan, Jakarta	15	21341
Ambulu, Besuki	20	18179
Palmerah, Jakarta	20	21390
Bagelen, Kebumen	25	36
Jatiroto, Pasuruhan	25	2959
Situbondo, Besuki	30	24460
Majenang, Banyumas	30-100	18433
Ngarengan, Juwana	50	34931
Banjar, Priangan	50	21355
Tegal, Pekalongan	50-100	3650
Balapulang, Slawi	50-100	15390
Jampang Kulon, Priangan	50-100	17408
Cikampek, Krawang	60	4596
Pati	75	634
Purwokerto, Banyumas	75	16
Banyumas	76	2311
Margasari, Pekalongan	90	195
Tulungagung, Kediri	100	11684
Mt. Pandan, Rembang	100	8

Ageratum conyzoides (continued)

Location	Altitude (m)	Herbarium number
Depok, Bogor	100	1171
Mt. Baluran, Besuki	100-200	24784
Jasinga, Bogor	100-250	10058
Solo	104	932
Purwakarta	110	52
Kresek, Madiun	125	1349
Rangkasbitung, Banten	125	2689
Banten	150-200	2030
Ciampea, Bogor	150	31476
Lebak, Pekalongan	150	4292
Karanganyar, Madiun	150	3
Surakarta	200	2844
Bogor	250	3816
Sukaraja, Kediri	250-500	22963
Pekalongan	300	15565
Pandeglang, Banten	300	7411
Krandegan, Madiun	300	2228
Cianjur	500-600	28
Priangan	500-600	16934
Salatiga, Semarang	570	30137
Temanggung	600	343
Kaliglidik, Pasuruhan	700	3783
Mt. Argopuro, Pasuruhan	800	13152
Dago, Bandung	800	47987
Mt. Salak, Cigombong	800-1000	9325
Jeluwang, Besuki	800-1600	11108
Nyalindung, Sukabumi	900-1000	14590

Ageratum conyzoides (continued)

Location	Altitude (m)	Herbarium number
Mt. Burangrang	1000	14255
Sepakung, Semarang	1000	35906
Cidadap, Cibeber	1000	2983
Mt. Ijen, Besuki	1100	24886
Garung, Kedu	1100	21983
Mt. Tengger	1100	23
Mt. Tengger, Pasuruhan	1200	88
Kediri	1300	113
Mt. Gede	1400	2942
Nirmala	1400-1500	20657
Lamongan, Besuki	1600	10756
Mt. Patuha	1750	1295
Dieng	1800	21759
Backer & Brink, 1965	0-2100	

Albizia lophantha

Ngebel, Ponorogo	1275	23154
	1450	3884
Pekalongan	1500	13348
Mt. Merbabu, Salatiga	1500	3885
Mt. Wilis, Kediri	1500	11396
Mt. Cikurai, Priangan	1500	5378
Mt. Ijen, Besuki	1550	24875
Mt. Lawu, Madiun	1650	6821
Mt. Papandayan	1800-2000	446
Mt. Merbabu, Surakarta	1800-2500	30258
Mt. Merapi, Surakarta	1900	97

Albizia lophantha (continued)

Location	Altitude (m)	Herbarium nuber
Mt. Anjasmara, Surabaya	1980	49
Dieng, Wonosobo	2000	21677
Tengger, Probolinggo	2000	37636
Dieng	2100	327
Mt. Sumbing, Kedu	2100	26
Mt. Gede	2135	513
Mt. Gede, Lebak Saat	2135-2600	535
Mt. Ijen, Besuki	2160	3892
Mt. Batok, Pasuruhan	2225	32633
Tengger, Probolinggo	2300	37632
Mt. Ijen, Besuki	2300	58
Priangan	2390	3717
Ngebel, Ponorogo	2400	3888
Mt. Arjuna	2400-3000	38139
Tengger, Probolinggo	2500	37635
Pasuruhan	2500	20
Mt. Cermai, Cirebon	2600-3075	5127
Mt. Merapi, Ijen	2600	43061
Mt. Pangrango	2600	31306
Mt. Papandayan	2650	11670
Mt. Slamet	2700-3100	500
Mt. Gede	2800	1999
Mt. Merbabu	2800	50
Mt. Kawi, Surabaya	2850	12232
Mt. Welirang, Besuki	2900	10954
Mt. Slamet	2900	3887
Mt. Arjuna	3000	43790

Albizia lophantha (continued)

Location	Altitude (m)	Herbarium number
Mt. Cermai	3000	2532
Mt. Argopuro	3020	43540
Mt. Lawi, Madiun	3100	2558
Mt. Merbabu	3125	1165
Top Mt. Welirang, Pasuruhan	3150	357
Top Mt. Arjuna, Pasuruhan	3300	331

Amaranthus spinosus

Panyawangan, Banten	1	1586
Pekalongan	5	15490
Tegal	5	15240
Puger, Besuki	10	18097 20447
Jakarta	10	31726 31705
Surabaya	10	4182
Jatiroto	20	7927
Kramatsentiong, Jakarta	20	8170
Majenang, Banyumas	30-100	18683
Asembagus, Besuki	30	24991
Situbondo	30	24488
Pasuruhan	50	2848
Juwana, Jepara	50	37183
Rangkasbitung, Banten	50	1002
Prajejan, Besuki	60	24559
Kediri	75	11253
Purwokerto	75 76	13 21032

Amaranthus spinosus (continued)

Location	Altitude (m)	Herbarium number
Jember	100	17731
Ciampea	150	31489
Rangkasbitung, Banten	150	2915
Besuki	200	22
Kediri	200	146; 64
Yogyakarta	200	2848
Kotaparis, Bogor	240	1152
Bogor	246 250	31572 1584
Kalisat, Besuki	250	18311
Sukaraja, Kediri	350-500	22886
Gondanglegi, Pasuruhan	450	3801
Ngebel, Ponorogo	700	23247
Bandung	700 800	518 1712
Garut	750	5184
Cibodas	1200	31897
Cisarua, Bogor	1200	20
Lembang, Bandung	1300	2461
Backer & Brink, 1963	1-1400	

Borreria brachystema

Northeast Java	150-500	(Backer & Brink, 1965)
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Cassia tora

Pekalongan	1	27419
Banten	1	1589

Cassia tora (continued)

Location	Altitude (m)	Herbarium number
Malingping, Banten	1	1385
	5	1427
Jakarta	5	435
Cempakaputih, Jakarta	5	32747
Puger	10-20	18202
Jakarta	15	32746
Mt. Madu, Banten	25	1666
Semarang	25-50	4171
Kebumen	30	122
Besuki	43	30677
Cililin, Banten	50-130	1103
Jepara	50	34870
	50	34869
Semarang	75	3955
Purwokerto	75	7
Jakarta	80	24062
Banjar, West Java	100	4385
Purwakarta	100	13969
Kediri	100	3178
Tulungagung	100	12035
Banten	100-150	2144
Wonosari, Jogjakarta	100-200	2550
Kediri	125	1545
		3227
Purbalingga	125	20
Cijujus, West Java	150	22505
Rembang	200	69887
Bogor	200	16627

Cassia tora (continued)

Location	Altitude (m)	Herbarium number
Pasuruhan	200-300	23913
Bogor	250	2087
Rajamandal	300	13474
Purwakarta	300	114
Gondanglegi, Banten	300	3877
Sukabumi	400	14855
Tengger	450-600	528
Malang	550	1899
Bandung	700	69742
Mt. Wilis, Ponorogo	850	23686

Cleome gynandra

West, Central and East Java	1-450	(Backer & Brink, 1963)
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Crotalaria mucronata

West, Central and East Java	1/2-1000	(Backer & Brink, 1963)
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Elaeagnus conforata

1000-1450	(Backer & Brink, 1965)
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Hypericum leschenaultii

Mt. Semeru	1160	7400
Juwana	1600-3000	340

Hypericum leschenaultii (continued)

Location	Altitude (m)	Herbarium number
Mt. Dworowati, Malang	1600	102
Mt. Gede, Cibodas	1650	2085
Mt. Ijen, Besuki	1750-2400	19
Mt. Wilis,	1800	949
Sembungan, Kedu	1800	70
Mt. Ijen, Besuki	1850	7
Mt. Merbabu	1900	84
Dieng	1900-2100	21631
Mt. Ungaran	2050	1241
Mt. Salak	2100	2998
Mt. Tengger, Pasuruhan	2200	510
Mt. Merapi, Surakarta	2200-2250	29
Mt. Argopuro	2200-3000	9732
Mt. Prah, Wonosobo	2300	303
Mt. Merbabu	2400-2500	30241
Mt. Papandayan	2400	280
Lalijiwo	2500	43849
Mt. Pangrango	2500	17608
Mt. Slamet	2700	485
Mt. Welirang, Pasuruhan	2760	7051
Mt. Cikurai	2800	8334
Mt. Ceremai	2800	12793
Mt. Pangrango	2800	2019
Mt. Ceremai	2950	5098
Mt. Slamet	3000	62022
Mt. Sindoro	3100	2918
Mt. Lawu, Madiun	3100	2551

Hypericum leschenaultii (continued)

Location	Altitude (m)	Herbarium number
Top Mt. Sumbing	3300	8738
Top Mt. Arjuna	3350	314

Hyptis brevipes

Kendal	2	16403
Malingping, Banten	5	1469
Brebes	5	15437
Batang	5	15532
Cirebon	20-30	6660
Puger	25	18267
Banyuwangi	25	120
Priangan	25	25647
Serang	25	13296
Mt. Madu, Banten	25	1670
Majenang	30	18514
Situbondo, Besuki	30	24425
Rangkasbitung	50	1004
Subah, Pekalongan	75	723
Purwokerto	75	89
Purwakarta	110	78
Lebak, Banten	125	2831
Banten	200	1870
Jampang-Kulon	200-250	17222
Bogor	240	1026
Jasinga, Bogor	250	10046
Tajur, Bogor	280	566
Baturaden	300	115

Hyptis brevipes (continued)

Location	Altitude (m)	Herbarium number
Bogor	300	2997
Kedu	380	39
Semarang	450-460	30168
Cianjur	500-600	245
Pesawahan, Priangan	500	25532
Mt. Karang, Banten	500	45
Temanggung	550	235
Cianjur	600	3080
Sukabumi	600-800	14626
Cibadak, Priangan	600	113
Wanayasa, Purwokerto	600	4837
Bandung	700	19
	800	1623
Ngadirejo	1000	692
Cadasmalang, Priangan	1000	1355
Cibeber	1000	73

Impatiens javensis

Kalibendo, Besuki	800	43251
Mt. Salak	800-1300	113973
Baturaden	900	39013
Pasirdatar, Pangrango	1000	2331
Mt. Tarub, Besuki	1000	10771
Cibeber	1000	619
	1100	22986
Nirmala	1200	10783
Mt. Halimun	1350	11187
Cibodas	1380	261

Impatiens javensis (continued)

Location	Altitude (m)	Herbarium number
Mt. Tikukur	1500	12877
Mt. Gede, Cibodas	1500	13532
Mt. Jang, Besuki	1500-1900	10815
Mt. Argopuro, Pasuruhan	1500	13108
Mt. Burangrang	1500	14024
Mt. Salak	1540	9298
Mt. Gede	1550	22278
Situ Lembang, Priangan	1577	63
Mt. Tangkubanperahu	1600	2437
Bandung	1600	11496
Mt. Ceremai	1600	5133
Mt. Gede	1600-1700	1427
Mt. Patuha	1600-2200	215
Mt. Mandalagiri	1650	248
Pengalengan	1650	26175
	1700	2369
Mt. Cikurai	1700	15025
Mt. Patuha	1750	1260
Mt. Cadaspanjang, Ciwidey	1750	1326
Mt. Burangrang	1790	4525
Garut	1800	135
Mt. Wayang	1900	317
Mt. Tangkubanperahu	1900	30908
Mt. Ceremai	2000	12819
Mt. Papandayan	2000	5579
Mt. Gede	2100	7735
Cibeureum	2300	2152
Backer & Brink, 1963	1000-2500	

Mycetia cauliflora

Location	Altitude (m)	Herbarium number
Mt. Tengger	800-1200	202
Mt. Gembung, West Java	1300	1201
Pasuruhan	1400	7334
Cibodas	1400	1843
	1400-1425	49
Mt. Sindoro	1650	116
Mt. Patuha	1700	1365
Backer & Brink, 1965	800-2200	

Myrsine affinis

Cemarlalawang, Pasuruhan	2100	38295
Mt. Arjuna, Pasuruhan	2100	38293

Polygonum chinense

From West to East Java	0-2800	(Koorders, 1912)
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Taraxacum officinale

Cisurupan, Priangan	1000	4039
Cisarua, Priangan	1200	66
Cikajang, Garut	1200	9592
Backer & Brink, 1965	1200-1500	
Lembang, Bandung	1250	
Mt. Papandayan	1300-1500	459
Mt. Cikurai, Priangan	1500	5431
Top Mt. Pangrango	3000	5026

4. SUMMARY

Cardinal temperatures of germination

1. The variation of the temperature span is mostly due to the variation of the maximum temperature of germination. Based on width and position of the temperature span, the thermal region of 14-29°C is favourable for seedling establishment of the Javanese species investigated. Species which are mostly found in higher altitudes show minimum and maximum temperatures of germination lower than 14°C and 29°C respectively. In contrary, species which are found or grow well in lowlands, have higher minimum and maximum temperatures of germination. Amaranthus paniculatus, Amaranthus caudatus, Phaseolus aureus and Sesbania bispinosa grow in tropical as well as temperate climates, they can germinate at temperature lower than 14°C and higher than 29°C.
2. Temperature minima and geographical or ecological distribution appeared to be correlated with Albizia lophantha, Polygonum chinense and Taraxacum officinale. This suggests that species with lower temperature minima germinate more effectively in low temperature of any thermal region than species with higher temperature minima. The extremely high maximum temperature of germination in Amaranthus spinosus, Sesamum orientale and Phaseolus pubescens demonstrates their ability to grow at the high temperatures in the lowland of dryer regions.
3. A significant correlation between optimum temperature of germination and mean altitude of plant distribution could be shown in non cultivated species grown in Java. It is suggested that the temperature optimum of germination is useful for the control of altitudinal distribution.

Germination at constant and alternating temperature

4. Temperature influences not only speed and percentage of germination, but can also affect the uniformity of germination. The distribution of germination in time of Ageratum conyzoides shows clearly a greater uniformity at higher temperature of germination.
5. Under darkness most species germinated well when submitted to alternating temperatures of $22/30^{\circ}\text{C}$. Positive response to alternating temperatures of $22/30^{\circ}$ and $10/30^{\circ}\text{C}$ was shown by Amaranthus spinosus. The relationship between position of optimum temperature of germination and effectiveness of alternating temperature shows that the closer the alternating temperature to the optimum temperature of germination, the more effective was the treatment.
6. The germination at constant and alternating temperature was not influenced by pre-imbibition treatment under light for 5 hours in the following species: Amaranthus spinosus, Amaranthus tricolor, Cassia tora, Phaseolus pubescens, Sesamum orientale, Sesbania bispinosa and Vigna unguiculata var. cylindrica. Positive response to pre-imbibition on germination was shown by Ageratum conyzoides, Talinum paniculatum and Amaranthus paniculatus. Light during pre-imbibition is needed for promotion in Ageratum conyzoides and Talinum paniculatum, but darkness is more effective for Amaranthus paniculatus. Germination of Cleome gynandra and Leucaena leucocephala was inhibited by pre-imbibition treatment; light inhibits germination.

Chilling and heat sensitivity of germination

7. In non cultivated species, chilling pretreatment at any temperature between $2-10^{\circ}\text{C}$ stimulates or promotes germination better

than in cultivated species. Chilling at $6.1-8^{\circ}\text{C}$ results in the highest chance for a promoted germination. A promoted response of germination to chilling treatment is stronger with species having low optimum temperature than with species having high optimum temperatures. In respect of its optimum temperature of germination, Amaranthus spinosus is similar to most cultivated species. By a chilling treatment at 10°C with or without pre-imbibition before germination, the seedling proportion of Amaranthus spinosus in crop population of Celosia argentea f. plumosa, Amaranthus caudatus, Amaranthus paniculatus, Sesbania bispinosa, Phaseolus pubescens and Phaseolus aureus becomes more competitive. Stratification at 14°C for 7 days increases the speed and uniformity of the germination response to temperature by Amaranthus spinosus.

8. Exposure to 40°C in moist condition before germination in normal temperature (26°C), produced a varied response. A promoted germination was shown by Leucaena leucocephala, Phaseolus aureus, Amaranthus spinosus, Cleome gynandra, Borreria brachystema, Sesbania bispinosa and Hyptis brevipes. Germination of some species is significantly inhibited by such a heating treatment: Amaranthus paniculatus, Hypericum leschenaultii, Impatiens javensis, Mycetia cauliflora and Cassia tora. An indifferent response of germination to a heating treatment for even 5 days was shown by Ageratum conyzoides, Crotalaria mucronata, Phaseolus pubescens, Sesamum orientale, Celosia argentea f. plumosa, Amaranthus tricolor, Talinum paniculatum and Ocimum americanum. A relation between heating resistance and mean altitude of distribution of non cultivated species from Java shows clearly that the higher the mean altitude, the weaker the heating resistance.

Storage conditions and longevity of seed

Germination ability at high and low temperatures was lost by storage for 106 days at 65°C in seeds (moisture content 7%) of Amaranthus paniculatus; a further effect was also the reduction of germination speed, resistance to chilling and heating treatment. To investigate the effect of a prestorage period on germinability and germination vigor, seed of Capsicum annuum was used as experimental material.

10. By applying the three viability equations (Roberts & Ellis, 1977), the viability constants of nine cultivated species have been determined. The influence of prestorage treatments on the value of K_{σ} has been investigated in seeds of Leucaena leucocephala, Phaseolus aureus and Sesamum orientale. For the different species, a relationship between the value of K_v and the period of viability is obvious. The higher the value of the K_v , the longer is period of viability (See tables 14 and 15, pages 73 and 74).

The longest viability may expected in the seeds of Amaranthus tricolor (955 years) for a decrease of 5% viability and the shortest viability in the seeds of Phaseolus aureus (12 years).

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6. REFERENCES

- ABDALLA, F.H. & E.H. ROBERTS, 1968. Effects of temperature, moisture, and oxygen on the induction of chromosome damage in seeds of barley, broad beans, and peas during storage. *Ann. Bot.* 32: 119-136.
- ABDALLA, F.H. & E.H. ROBERTS, 1969. The effects of temperature and moisture on the induction of genetic changes in seeds of barley, broad beans and peas during storage. *Ann. Bot.* 33: 153-167.
- ASAKAWA, S., 1959. Physiology of embryo dormancy. *J. Jap. For. Soc.* 41: 389-395.
- BACKER, C.A. & R.C.B. v.d. BRINK, 1963. Flora of Java
1. N.V.P. Noordhoff, Groningen.
- BACKER, C.A. & R.C.B. v.d. BRINK, 1965. Flora of Java
2. N.V.P. Noordhoff, Groningen.
- BARON, F.J., 1970. Metabolic patterns in dormant and germinating seeds of sugar pine. *Adv. Frontiers Plant Sci.* 24: 47-64.
- BARTON, L.V., 1961. Seed preservation and longevity.
Leonard Hill, London.
- BARTON, L.V., 1965. Seed Dormancy: Encyclopedia of Plant Physiology
15(2): 699-717(a). 727-742(b).
- BASKIN, J.M. & C.C. BASKIN, 1977. Germination ecology of
Sedum pulchellum. *Amer. J. Bot.* 64: 1242-1247.
- BLAKESLEE, A.F. & A.G. AVERY, 1934. Visible genes from aged seeds. *Amer. Nat.* 68: 466.
- BRAAK, C., 1945. On the Climate of and Meteorological research in the Netherlands Indies. In: Science and Scientists in the Netherlands Indies, P. HONIG & F. VERDOORN, Eds. Board for the Netherlands Indies, New York City, Surinam and Curacao: 15-22.
- CARTLEDGE, J.L. & A.F. BLAKESLEE, 1933. Mutation rate increased by ageing seeds as shown by pollen abortion. *Science* 78: 523.

- CARTLEDGE, J. L. & A. F. BLAKESLEE, 1934. Mutation rate increased by ageing seeds as shown by pollen abortion. *Proc. natn. Acad. Sci. USA.* 20: 103-110.
- CARTLEDGE, J. L., L. V. BARTON & A. F. BLAKESLEE, 1936. Heat and moisture as factors in the increased mutation rate from Datura seeds. *Proc. Am. phil. Soc.* 76: 663-685.
- CHATTERJI, U. N. & K. MOHNOT, 1968. Ecophysiological investigations on the imbibition and germination of seeds of Prosopis juliflora Linn. In: *Proceedings of the Symposium on Recent Advances in Tropical Ecology, Part I*, R. MISRA & B. GOPAL Eds. The International Society for Tropical Ecology, Varanasi: 261-268.
- CHOATE, H. A., 1940. Dormancy and germination in seeds of Echinocystis lobata. *Amer. J. Bot.* 27: 156-160.
- COHEN, D., 1958. The mechanism of germination stimulation by alternating temperatures. *Bull. Res. Coun. Israel* 6d: 111-117.
- DAVIS, W. E. & R. C. ROSE, 1912. The effect of external conditions upon the after-ripening of the seeds of Crataegus mollis. *Bot. Gaz.* 54: 49-62.
- DE LA ROCHE, I. A., C. J. ANDREWS & M. KATES, 1973. Changes in phospholipid composition of winter wheat cultivar during germination at 2°C and 24°C. *Plant Physiol.* 51: 468-473.
- DOGRAS, C. C., D. R. DILLEY & R. C. HERNER, 1977. Phospholipid Biosynthesis and Fatty Acid Content in Relation to Chilling Injury during germination of seeds. *Plant Physiol.* 60: 897-902.
- ECKERSON, S., 1913. A physiological and chemical study of after-ripening. *Bot. Gaz.* 55: 286-299.
- EVENARI, M., 1965. Light and Seed Dormancy. *Encyclopedia of Plant Physiology* 15 (2): 804-841.
- FLEMION, F., 1931. After-ripening germination and vitality of seeds of Sorbus aucuparia L. *Contrib. Boyce Thompson Inst.* 3: 413-439.
- FRECHT, H., J. CHRISTOPHERSEN, H. HENSEL & W. LARCHER, 1973. *Temperature and life*. Springer-Verlag, Berlin, Heidelberg, New York.

- GOOD, R., 1964. The Geography of the Flowering Plants. Longmans, Green and Co Ltd, London.
- GULLIVER, R. L. & W. HEYDECKER, 1973. Establishment of Seedling in a changeable environment. In: Seed Ecology, W. HEYDECKER, Ed. Butterworths, London: 433-462.
- GUNTARDT, H., L. SMITH, M. E. HAFERKAMP & R. A. NILAN, 1953. Studies on aged seeds II. Relation of age of seeds to cytogenic effects. Agron. J. 45: 438-441.
- HAUT, I. C., 1933. Effect of various low temperatures on fruit tree seeds. Proc. Amer. Soc. Hort. Sci. 30: 365-367.
- HILL, T. A., 1977. The Biology of Weeds. The Institute of Biology's Studies in Biology no. 79. Edward Arnold, London.
- HOELZL, J. & H. WAGNER, 1971. A method for preparing radioactive labeled phosphatides using soybeans in vivo. Z. Naturforschung 26: 425-434.
- JAMES, E., 1967. Preservation of seed stocks. Adv. Agron. 19: 87-106.
- KOORDERS, S. H. 1912. Exkursionsflora von Java 3. Gustav Fischer, Jena.
- LANG, A., 1965. Effects of Some Internal and External Conditions on Seed Germination. Encyclopedia of Plant Physiology 15 (2): 848-886.
- MAUN, M. A. & P. B. CAVERS, 1971. Seed production and dormancy in Rumex crispus I. The effects of removal of cauline leaves at anthesis. Canadian Journal of Botany 49: 1123-1130.
- MITCHELL, R. L., 1970. Crop growth and culture. The Iowa State University Press & Ames.
- MORINAGA, T., 1926. Effect of alternating temperatures upon the germination of seeds. Am. J. Bot. 13: 141-158.
- NAVASHIN, M. S., 1933a. Origin of spontaneous mutations. Nature, Lond. 131: 436.
- NAVASHIN, M. S., 1933b. Ageing of seeds is a cause of chromosome mutations. Planta 20: 233-243.

- NAVASHIN, M.S. & H.N. GERASSIMOVA, 1936a. Natur und Ursachen der Mutationen I. Das Verhalten und die Zytologie der Pflanzen, die aus infolge Alterns mutierten Keimen stammen. *Cytologia* 7: 324-362.
- NAVASHIN, M.S. & H.N. GERASSIMOVA, 1936b. Natur und Ursachen der Mutationen. III. Ueber die Chromosomenmutationen, die in den Zellen von ruhenden Pflanzenkeimen bei deren Altern auftreten. *Cytologia* 7: 437-465.
- NICHOLS, C., 1941. Spontaneous chromosome aberration in *Allium*. *Genetics* 26: 89-100.
- NIKOLAEVA, M.G., 1969. Physiology of Deep Dormancy in Seeds. Israel Program for Scientific Translations Ltd., Jerusalem.
- OWEN, E.B., 1956. The storage of seeds for maintenance of viability. Commonwealth Agricultural Bureaux. Farnham Royal, England.
- OXLEY, T.A., 1948. The Scientific Principles of Grain Storage. Northern Publishing Co., Liverpool.
- PANDEY, S.B., 1968. Germination and Seedling Mortality in *Echinops echinatus* Roxb. In: Proc. Symp. Recent Adv. Trop. Ecol., R. MISRA & B. GOPAL Eds. The International Society for Tropical Ecology, Varanasi, India: 237-243.
- PETO, F.H., 1933. The effect of ageing and heat on the chromosomal mutation rate in maize and barley. *Can. J. Res.* 9: 261-264.
- POLLOCK, B.M. & H.O. OLNEY, 1959. Studies of the rest period. I. Growth, translocation, and respiratory changes in the embryonic organs of the after-ripening cherry seed. *Plant Physiol* 34: 131-142.
- POLLOCK, B.M. & V.K. TOOLE, 1966. Imbibition period as a critical temperature sensitive stage in germination of lima bean seeds. *Plant Physiol.* 41: 221-229.
- ROBERTS, E.H., 1960. The viability of cereal seed in relation to temperature and moisture. *Ann. Bot.* 24: 12-31.
- ROBERTS, E.H., 1961. Viability of cereal seed for brief and extended periods. *Ann Bot.* 25: 373-380.

- ROBERTS, E.H., 1972. Storage Environment and the Control of Viability. In: Viability of Seeds, E.H. ROBERTS, Ed. Chapman and Hall Ltd., London: 14-58.
- ROBERTS, E.H., F.H. ABDALLA & R.J. OWEN, 1967. Nuclear damage and the ageing of seeds with a model for seed survival curves. Symp.Soc.exp.Biol. 21: 65-100.
- ROBERTS, E.H. & F.H. ABDALLA, 1968. The influence of temperature, moisture, and oxygen on period of seed viability in barley, broad bean, and peas. Ann.Bot.: 32: 97-117.
- ROBERTS, E.H. & R.H. ELLIS, 1977. Prediction of seed longevity at sub-zero temperatures and genetic resources conservation. Nature 268: 431-433.
- ROBERTS, H.F., 1924. Germination of seeds exposed to low temperature. Nature (Lond.) 114: 393.
- SAX, K. & H.J. SAX, 1964. The effect of chronological and physiological ageing of onion seeds on the frequency of spontaneous and X-ray induced chromosome aberrations. Radiat. Bot. 4: 37-41.
- SHETTY, M.S., 1968. Germination behaviour of the seeds of Biophytum sensitivum (L.) DC. In: Proc. Symp. Recent. Adv. Trop. Ecol. Part I., R. MISRA & R. GOPAL, Eds. The International Society for Tropical Ecology. Varanasi, India: 213-224.
- SHEWRY, P.R., N.J. PINFIELD & A.K. STOBART, 1973. Phospholipids and the phospholipid fatty acids of germinating hazel seeds (Corylus avellana L.) J.Exp.Bot. 24: 1100-1105.
- STONE, E.C., 1957. Embryo dormancy of Pinus jeffreyi Murr. seed as affected by temperature, water uptake, stratification and seed coat. Plant Physiol. 32: 93-99.
- STRAFFORD, G.A., 1965. Essentials of Plant Physiology. Heinemann Educational Books Ltd., London.
- SUTCLIFFE, J., 1977. Plants and Temperature. Edward Arnold Ltd., London.
- THOMPSON, K., J.P. GRIME & G. MASON, 1977. Seed germination in response to diurnal fluctuations of temperature. Nature 267: 147-149.

- THOMPSON, P.A. , 1968. Germination of Caryophyllaceae at Low Temperatures in relation to Geographical Distribution. *Nature* 217: 1156-1157.
- THOMPSON, P.A. , 1970. Characterization of the germination response to temperature of species and ecotypes. *Nature* 225: 827-831.
- THOMPSON, P.A. , 1974a. Germination of Celery (*Apium graveolens* L.) in Response to Fluctuating Temperatures. *J. Exp. Bot.* 25 (84): 156-163.
- THOMPSON, P.A. , 1974b. Effects of Fluctuating Temperatures on Germination. *J. Exp. Bot.* 25(84): 164-175.
- VEGIS, A. , 1964. Dormancy in higher plants. *Ann. Rev. Plant Physiol.* 15: 185.
- VILLIERS, T.A. , 1975. Dormancy and the survival of plants. Edward Arnold, London.
- VINCENT, E.M. & P.B. CAVERS, 1978. The effects of wetting and drying on the subsequent germination of *Rumex crispus*. *Can. J. Bot.* 56: 2207-2217.
- WAREING, P. F. , 1965. Endogenous inhibitors of seed germination and dormancy. *Encyclopedia of plant physiology* 15(2): 909-922.
- WEBER, E. , 1972. *Grundriss der Biologischen Statistik*. Gustav Fischer Verlag, Stuttgart.
- WENT, F.W. , 1957. Experimental control of plant growth. *Chronica Botanica*, Waltham Mass.
- WILLEMSSEN, R.W. , 1975. Effect of stratification temperature and germination temperature on germination and the induction of secondary dormancy in common ragweed seeds. *Amer. J. Bot.* 62 (1): 1-5.
- WOODSTOCK, L.W. & B.M. POLLOCK, 1965. Physiological pre-determination: Imbibition, Respiration, and growth of lima bean seeds. *Science* 150: 1031-1032.

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